NOTICES *

This document has been translated by computer. So the translation may not reflect the original precisely.
**** shows the word which can not be translated.
In the drawings, any words are not translated.

CLAIMS

Claim(s)]
Claim 1]N-acetyl glucosamine transfer enzyme which has the following physicochemical property.
ctivity: Transfer N-acetyl glucosamine to galactose residue which exists in a nonreducing terminal of an N-etyl glucosamine receptor sugar chain by alpha 1 and 4 combination from an N-acetyl glucosamine donor.
Claim 2]An N-acetyl glucosamine donor is a sugar nucleotide which has N-acetyl glucosamine residue, an N-etyl glucosamine receptor -- the galactose Y (however, - a glycosidic linkage.) Y shows N-acetyl hexosamin
/hich a sugar chain which has N-acetyl hexosamine and N-acetyl hexosamine in a nonreducing terminal, or glycon combined -- it is -- the N-acetyl glucosamine transfer enzyme according to claim 1.
Claim 3]The N-acetyl glucosamine transfer enzyme according to claim 1 or 2 which has the further following ysicochemical property.
n N-acetyl-glucosamine donor: UDP-N-acetylglucosamine (UDP shows uridine diphosphate)
ubstrate specificity: Transfer N-acetyl glucosamine to galactose residue of a nonreducing terminal of a eceptor of either the following ** - ** from an N-acetyl glucosamine donor.
' Galbeta1, 3 GalNAcalphapNP**GlcNAc beta 1, 6. (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, lcNAcbeta1, 6 (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, 4 GlcbetapNP**Gal beta 1, 3GlcNAcbetapNP among a formula) Glucose residue and GalNAc show N-acetyl galactosamine residue, GlcNAc shows N-etyl glucosamine residue, and, as for Gal, pNP shows p-nitrophenol residue, as for galactose residue and lc.
ctivation and inhibition: Activity is promoted by Mn^{2+}. Activity is controlled by EDTA.
Claim 4]The following (a) or polypeptide of (b).
a) Polypeptide which includes an amino acid sequence of the amino acid numbers 96-340 in an amino acid equence of the array number 2 at least.
or some amino acid replace in an amino acid sequence of polypeptide of (b) and (a), Consist of deletion and amino acid sequence inserted or transferred, and from an N-acetyl glucosamine donor. Polypeptide which onstitutes an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4

ombination, or has the same antigenicity as polypeptide of (a).

Claim 5]Including a part of amino acid sequence of the array number 2 from an N-acetyl glucosamine donor. 'olypeptide which constitutes an enzyme which has the activity which transfers N-acetyl glucosamine to lactose residue which exists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by pha 1 and 4 combination, or consists of an amino acid sequence of the array number 2, and polypeptide /hich has the same antigenicity.

Claim 6]DNA which encodes polypeptide which constitutes N-acetyl glucosamine transfer enzyme which has ıe activity which transfers N-acetyl glucosamine to galactose residue which exists in a nonreducing terminal an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 combination from an N-acetyl glucosamine nor.

Claim 7]The DNA according to claim 6 which has a physicochemical property of the following [transfer zyme / N-acetyl glucosamine].

ın N-acetyl-glucosamine donor: UDP-N-acetylglucosamine (UDP shows uridine diphosphate)

ubstrate specificity: Transfer N-acetyl glucosamine to galactose residue of a nonreducing terminal of a eceptor of either the following ** - ** from an N-acetyl glucosamine donor.

' Galbeta1, 3 GalNAcalphapNP**GlcNAc beta 1, 6. (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, lcNAcbeta1, 6 (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, 4 GlcbetapNP**Gal beta 1, 3GlcNAcbetapNP among a formula) Glucose residue and GalNAc show N-acetyl galactosamine residue, GlcNAc shows N-etyl glucosamine residue, and, as for Gal, pNP shows p-nitrophenol residue, as for galactose residue and ilc.

ctivation and inhibition: Activity is promoted by Mn^{2+}. Activity is controlled by EDTA.

Claim 8]DNA which encodes polypeptide of the following (a) or (b).

a) Polypeptide which includes an amino acid sequence of the amino acid numbers 96-340 in an amino acid equence of the array number 2 at least.

or some amino acid replace in an amino acid sequence of polypeptide of (b) and (a), Consist of deletion and amino acid sequence inserted or transferred, and from an N-acetyl glucosamine donor. Polypeptide which onstitutes an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination, or has the same antigenicity as polypeptide of (a).

Claim 9]Including a part of amino acid sequence of the array number 2 from an N-acetyl glucosamine donor. . hether an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination is constituted, and] Or DNA which encodes polypeptide which consists of an amino acid equence of the array number 2, and polypeptide which has the same antigenicity.

Claim 10]The DNA according to claim 8 which is the polypeptide which polypeptide of (a) turns into from an ino acid sequence of the array number 2.

Claim 11]DNA which has a base sequence of the base numbers 181-1200 in a base sequence of the array

mber 1.

Claim 12]Polynucleotide hybridized to DNA which has a base sequence complementary to DNA claim 6 - ven in 11 any 1 paragraphs, or a base sequence of the DNA.

Claim 13]A recombinant vector containing DNA claim 6 - given in 11 any 1 paragraphs.

Claim 14]A transformant which DNA claim 6 - given in 11 any 1 paragraphs is introduced, and can reveal this)NA.

Claim 15]A transformant containing the recombinant vector according to claim 13.

Claim 16]Cultivate the transformant according to claim 14 or 15 by a culture medium, and generation cumulation of the N-acetyl glucosamine transfer enzyme containing polypeptide or it which said DNA codes is carried out into a culture, A manufacturing method of N-acetyl glucosamine transfer enzyme ontaining polypeptide or it extracting N-acetyl glucosamine transfer enzyme which contains polypeptide or it om the culture.

Claim 17]Detection system of gastric cancer or a pancreatic cancer relating an expression amount, and stric cancer or a pancreatic cancer of DNA of a statement with any 1 paragraph of claims 6-12 in body fluid xtracted from a living body.

Claim 18]The detection system according to claim 17 whose body fluid is blood.

Claim 19]A diagnostic kit of gastric cancer or a pancreatic cancer by which an oligonucleotide for detecting a anifestation of DNA of a statement being included in any 1 paragraph of claims 6-12.

Claim 20]The diagnostic kit according to claim 19 containing reverse transcriptase and DNA polymerase.

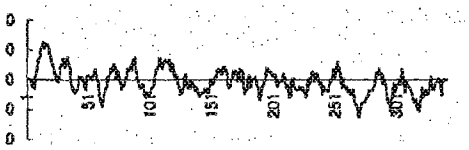
Translation done.]

JAPANESE [JP,2001-046077,A]

LAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION ECHNICAL PROBLEM MEANS EXAMPLE DESCRIPTION OF DRAWINGS DRAWINGS CORRECTION OR MENDMENT

Translation done.]

Drawing selection Drawing 1



[Translation done.]

NOTICES *

This document has been translated by computer. So the translation may not reflect the original precisely.
**** shows the word which can not be translated.
In the drawings, any words are not translated.

EXAMPLE

Example]Hereafter, although this invention is concretely explained in full detail according to an example, as fo
his invention, limitation is not carried out to this.

0088]1. 30micro of cDNA library [of a human stomach organization] (made by Clontech) g included in DNAI which is an expression vector of the screening eukaryotic cell of the cell which reveals this invention zyme, It introduced into COS-1 cell of the $1.2x10^7$ individual with pRcCMV-leu 30microg which reveals the man leuco sialin. After culturing this COS-1 cell for 60 hours, COS-1 cell which revealed the GlcNAc residue ombined with the surface of the cell by alpha combination at the nonreducing terminal of the sugar chain, the tibody mixed liquor (HIK1083, PGM36, and PGM37 (Biochem. J. 318 and 409-416.)) which reacts to the cel orter (FACStar) by BEKUTON Dickinson, and GlcNAc of which alpha combination was done specifically from 96, Comp. Biochem. Physiol. 121B, 315-321, and 1998 -- becoming -- it condensed by used fluorescence tivation cell sorting (FACS), and 258 COS-1 cells were collected.

0089]Then, plasmid DNA was collected in accordance with the conventional method, and it introduced into E. oli MC1061/P3 which are a host bacterium using Cell-Porator (made by a life technology company). 325 olonies produced the host bacterium which contains the target cDNA among the introduced host bacteria in election by the tolerance over ampicillin and a tetracycline.

0090]325 colonies were transferred to the nitrocellulose membrane, the replica was created, this replica was vided equally ten, and was pooled, and plasmid DNA was collected from each pool. The obtained plasmid NA was again introduced into COS-1 cell with pRcCMV-leu, the immunofluorescenct stain which uses above ientioned antibody mixed liquor was performed, and COS-1 cell was observed under the microscope after iat. As a result, existence of COS-1 cell which shows a strong fluorescence was checked, and the host in /hom the colony which held the target cDNA via the corresponding replica exists was specified.

0091]A new replica was created like the above from this specified host, and the host who holds the target DNA like the above was narrowed down. When creation of a replica - the host's narrowing down were epeated 3 times and performed, it became one colony which exists in a host. The plasmid which the host cterium in which this colony was formed held was named pcDNAI-alpha 4GnT.

0092]2. Plasmid DNA is collected from the colony of the host bacterium narrowed down by determination 1. o

ıe base sequence in accordance with a conventional method, By the DAIJIDEOKISHI nucleotide chain ermination (dye dideoxy nucleotide chain-termination) method which uses the 373A DNA sequencer made om applied bio-cis- TEMUSU with a conventional method, 3'->5', The base sequence was analyzed to rection of both 5'->3'. As a result, it became clear that cDNA of alpha4GnT which has a base sequence of th ray number 1 statement which consists of a base pair of 1292bp was inserted in the plasmid DNA included i ıis host bacterium. The amino acid sequence (array number 2) which consists of 340 amino acid residue was edicted from the open reading frame of this base sequence.

)093]From this amino acid sequence, a molecular weight is 39,497Da and the protein which has four parts hey are the amino acid numbers 99, 138, 251, and 282 in the array number 2) which N-knot-pattern sugar ain may combine was predicted. From the hydropathy plot (drawing 1) created from this amino acid equence. One remarkable hydrophobic part which length 22 residue over the 4-25th amino acid residue)llowed from the amino terminal is accepted, The amino terminal side of the portion was short, and since the rtion was sandwiched by basic-amino-acid residue, it was expected that alpha4GnT is film penetration otein of Type II which has a transmembrane domain.

)094]3. Plasmid vector pcDNAI-alpha 4GnT was prepared in accordance with the conventional method from ıe colony of the host bacterium obtained by manifestation 1. in COS-1 cell of alpha4GnT. This plasmid vector pcDNAI (contrast) was introduced into COS-1 cell with pRcCMV-leu. Similarly, pcDNAI-alpha 4GnT was ıdependently introduced into COS-1 cell. Fix each cell 60 hours after introduction, make it react to antibody IIK1083 or anti-leuco sialin antibody 1G10 which reacts to GlcNAc of which alpha combination was done pecifically (PharMingen), and it ranks second, It was made to react to anti-mouse IgM (as opposed to IIK1083) or anti-mouse IgG (as opposed to 1G10) which combined the fluorescein isothiocyanate.

)095]Dyeing was not carried out by HIK1083 although immunity dyeing of the COS-1 cell which introduced cCMV-leu and pcDNAI was carried out by 1G10. Immunity dyeing of the COS-1 cell which introduced cCMV-leu and pcDNAI-alpha 4GnT was carried out by both 1G10 and HIK1083. COS-1 cell which ıtroduced only pcDNAI-alpha 4GnT was not dyed by 1G10, but the weak dye affinity was seen in HIK1083. his has suggested that HIK1083 combines with alpha 1 and 4-GlcNAc which were added to cluster O-glycan ıore strongly. Also when immunity dyeing was carried out by each of PGM36 and PGM37, the same dye finity as the case where immunity dyeing is carried out by HIK1083 was seen.

)096]Participating in the activity which adds GlcNAc to the nonreducing terminal of the mucin type sugar chai hich has protein by which a code is carried out to pcDNAI-alpha 4GnT on the leuco sialin from these results alpha combination was shown.

)097]4. Since it was predicted from amino acid sequence of alpha4GnT predicted from preparation base equences of DNA which encodes alpha4GnT of soluble gestalt which has partial sequence of alpha4GnT tha pha4GnT is membrane protein of Type II, alpha4GnT (it is indicated also as the following "alpha4GnT (S)") o ıe soluble gestalt which carried out deletion of the transmembrane domain was prepared. . Namely, carried t deletion of the amino acid of the N terminal region equivalent to the amino acid numbers 1-27 of the amino id sequence of array number 2 statement. In order to obtain alpha4GnT (S) which has an amino acid

equence of the amino acid numbers 28-340 in the amino acid sequence of the array number 2, The primer /hich has a base sequence of array number 3 statement An upstream region primer (it has a BamHI site ıside), The PCR method was performed by using pcDNAI-alpha 4GnT as a mold, using the primer which has base sequence of array number 4 statement as a downstream area primer (it has a XhoI site inside).)igestive treatment of the acquired PCR product was carried out with the restriction enzymes BamHI and :hoI, and DNA which has a base sequence of the base numbers 262-1215 in the base sequence of array mber 1 statement was prepared. Protein A. DNA to encode. The base sequence of the above-mentioned se numbers 262-1215 to the BamHI-XhoI site of the incorporated pcDNAI plasmid vector (pcDNAI-A) (J. iol.Chem. 274, 3215-3221, 1999). DNA which it has was incorporated and pcDNAI-A-alpha 4GnT (S) was btained. Since this plasmid vector encodes alpha4GnT (S) and protein A to the same read-out field, it reveals ıe fused protein (alpha4GnT(S)-A) of alpha4GnT (S) and protein A.

)098]5. In accordance with refining of alpha4GnT (S), and a substrate specificity examination conventional ıethod, pcDNAI-A-alpha 4GnT (S) is introduced into COS-1 cell, It was isolated from the culture supernatant ing the IgG-Sepharose column (Amersham Pharmacia manufacture) like the method given [alpha4GnT(S)-] in J. Biol. Chem. 274, 3215-3221, and 1999.

)099]Although the substrate specificity examination was done according to the method of J. Biol. Chem. 274, 15-3221, and the substrate specificity examination indicated to 1999, the activity of this invention enzyme /as controlled [three] by EDTA of 10 mM in about 2/. Since the activity of this invention enzyme was omoted about 1.3 times by adding Mn^{2+} of 5 mM, without adding EDTA, In said method, alpha4GnT activity /as measured as a receptor of N-acetyl glucosamine using the receptor synthesis substrate indicated in Table by the method which replaced reaction mixture with the reaction mixture which contains $MnCl_2$ of 5 mM ıstead of EDTA. Receptor synthesis substrate No. of Table 1 The result which made activity over 9 100% anc /as expressed with the relative activity over other substrates is shown in drawing 2.

)100]

Table 1]

表 1

	受容体合成基質	入手方法
1	Galαp NP	シグマ社より購入した。
2	Galβp NP	
3	Galβ1,3GlcNAcβp NP	
4	Galβ1,4Glcβp NP	
5	GalNAcαp NP	
6	Galβ1,3GalNAcαp NP	
7	GlcNAcβ1,3GalNAcαp NP	トロントリサーチケミカルス社より購入した。
8	GlcNAcβ1,6(Galβ1,3)GalNAcαp NP	
9	Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcαp NP	J.Biol.Chem.273,34843,1998 に従って、8 を使用して合成した。

)101]As shown in drawing 2, alpha4GnT (S) became clear [that the activity which transfers GlcNAc is the trongest] to Galbeta1 which is branched oligosaccharides of the core 2, 4GlcNAcbeta1, and 6(Galbeta1, 3)

GalNAcalphapNP (Table 1, No. 9). Rather than Galbeta1 and 3GalNAcalphapNP (Table 1, No. 6) which are ne receptor synthesis substrates of the core 1 as for alpha4GnT (S). The result which shows that the activity hich transfers GlcNAc to GlcNAcbeta1 and 6(Galbeta1, 3)GalNAcalphapNP (Table 1, No. 8) more is strong as obtained.

0102]Although alpha4GnT (S) transfers GlcNAc more to Galbeta1 and 3GalNAcalphapNP (Table 1, No.6) as ompared with Galbeta1 and 4GlcbetapNP, The activity which furthermore transfers GlcNAc to GlcNAcbeta1, Galbeta1, 3)GalNAcalphapNP (Table 1, No. 8) and Galbeta1, 4GlcNAcbeta1, and 6(Galbeta1, 3) GalNAcalphapNP (Table 1, No. 9) is strong. It had the weak activity which transfers GlcNAc to Galbeta1, lcNAcbetapNP (Table 1, No. 3) and Galbeta1, and 4GlcbetapNP (Table 1, No. 4). It was surmised that pha4GnT (S) mainly recognized the galactose residue united by beta 1 in O-glycan and 4 combination and ta 1, and 3 combination from this, and GlcNAc was transferred. alpha4GnT (S) did not transfer sugar GalNAc) to which above-mentioned receptor synthesis substrate, when UDP-GalNAc was used as a sugar nor.

0103]Since the structure of the output produced by the GlcNAc transition activity of alpha4GnT (S) is pecified, The Sep-Pak C18 cartridge column was used and the resultant which scaled up reaction mixture to 0microl on above-mentioned conditions, made react overnight, and was acquired was refined in accordance ith J. Biol. Chem.273, 34843-34849, and the method indicated to 1998. After checking by HPLC that the rity of a resultant is not less than 99%, in accordance with the conventional method, NMR analyzed tructure. The analysis of the NMR spectrum used the 500 MHz Varian Unity-Plus spectrometer. All the pectra of a receptor and alpha-D-GalNAc-pNP, Galbeta1, 3 GalNAc-pNP and Galbeta1, and 3 (GlcNAcbeta1 GalNAc-pNP were used for attribution of a chemical shift. The spectrum measured heavy water as a olvent, after performing repetition heavy water substitution. The spectrum of ^{1}H NMR belonged combining the wo-dimensional 2 quantum filter correlation spectrum (2D-DQF-COSY) and the two-dimensional total orrelation spectrum (2D-TOCSY:50 ms). In order to raise accuracy, spectrum analysis was conducted at 5 **, **, and 30 **. It opted for attribution and the glycosidic linkage of ^{13}C, using a different core multiplex antum coherence spectrum (HMQC) and a two-dimensional rolling-mechanism NOE spectrum (2D-ROESY: 0 ms and 300 ms) auxiliary.

0104]The structural analysis by NMR was conducted using the resultant to which GlcNAc was transferred by ne activity of alpha4GnT (S) by making Galbeta1, 4GlcNAcbeta1, and 6(Galbeta1, 3)GalNAcalphapNP 300- nomole into a receptor. As shown in Table 2, the specific signal of the double line was obtained from the pectrum of ^{1}H NMR about the atom of six anomers. The signal of the non-anomer proton belonged based on ne crossing peak observed by the position of H-4 of 2D-DQF-COSY and a 2D-TOCSY spectrum. The pectrum of ^{13}C NMR was analyzed by two-dimensional hydrogen detection different core ^{1}H-^{13}C correlation hich uses reverse mode. As for the carbon atom of the 4th place of beta-D-Gal residue, it was shown by the HMQC spectrum that substitution has taken place. In the 2D-ROESY spectrum, the crossing peak near alpha-)-GlcNAc H-1-/beta-D-Gal H-4delta3.96 ppm was observed, and it was shown that it has joined together by

pha 1 and 4 combination. Other crossing peaks in a spectrum agreed in the structure which GlcNAc ombined with Gal by alpha 1 and 4 combination.

)105]

Table 2]

表 2

糖	H-1	C-1	H-2	C-2	H-3	C-3	H-4	C-4	H-6	C-6
α-D-GalNAc	5.8 (3.5Hz)	97.5	4.6	49.7	4.35	78.0	4.25	70.5	3.57	--
2xα-D-GlcNAc	4.87 (3.5Hz)	99.8	3.89	54.7	3.78	71.8	3.54	70.2	--	--
	4.88 (3.5Hz)	99.9								
2xβ-D-Gal	4.59 (7Hz)	105.5	3.56	71.2	3.72	72.1	3.96	78.3	--	--
	4.42 (8Hz)	104.9	3.58		3.70		3.95			
β-D-GlcNAc	4.44 (8Hz)	102.5	3.56	56.0	3.62	71.8	3.74	76.2	--	--

-は測定せず。

)106]6. alpha4GnT manifestation 6-1 in each organization cDNA of alpha4GnT which started from NOZAN ot analysis pcDNAI-alpha 4GnT, and isolated by gel electrophoresis, The label was carried out by [alpha^{32}P] TP using the Stratagene Prime-It II labeling kit, and the radioactive probe was created (1x10 ^{6}cpm/ml). The bove-mentioned probe, The Clontech membrane for Homo sapiens multi-organization NOZAN blots (healthy ult organization) and (Multiple Tissues Nothern Blots), the Clontech hybridization solution (ExpressHyb.) It alyzed according to the manual attached to the kit using Hybridization Solution. Namely, ExpressHyb lybridization Solution is heated at 68 **, Multiple Tissues Nothern Blots was shaken in 30 minutes and in bove-mentioned ExpressHyb Hybridization Solution at 68 **, and pre hybridization was performed. Above- ıentioned Multiple Tissues Nothern Blots was shaken at 68 ** for 1 hour in ExpressHyb Hybridization Solutior ml which denatures the above-mentioned probe for 2 minutes at 95 **, quenches, and contains this probe ter that. This film was shaken at the room temperature for 30 minutes in the penetrant remover, and it shook ı the penetrant remover for 40 minutes at 50 more **. The X-ray film was exposed with autoradiography using ultiple Tissues Nothern Blots which the radioactivity probe prepared in this way combined. As a result, the anscript of the alpha4GnT gene of the size of 1.7kb was observed in the stomach and the pancreas. urthermore, the transcript of 2.1kbs and 0.7kbs was faintly observed by the stomach and the pancreas.

)107]6-2 In order to detect GlcNAcalpha1 in the manifestation human tissue in the organization of ;lcNAcalpha1, 4Gal beta->R structure, or III type mucus, and the manifestation of 4Gal beta->R structure, Th nmunostaining method which uses HIK1083 antibody was performed in accordance with Histochem.Cell Biol 0, 113-119, 1998 and J. Histochem. Cytochem. 46, 793-801, and the method indicated to 1998. That is, ter [which was chosen from the pathology section file of Shinshu University Hospital] formalin fixation was arried out, the Homo sapiens healthy tissue sample (the stomach, the pancreas, and large intestine) by whicl raffin embedding was carried out was attached to non-aqueous methanol containing 0.3% of hydrogen

roxide solution for 30 minutes. The immunity tissue staining color by HIK1083 antibody used the adjective taining method (Ann. NY Acad. Sci. 254, 203-211, 1975). As a second antibody, the goat antimouse nmunoglobulin antibody which combined peroxidase (made by DAKO) of the horseradish was used, and it ras made to color using a diaminobenzidine hydrogen-peroxide-solution solution. The chromatic figure strong gainst the gland pipe epithelium which showed the accessory gland and gastric metaplasia of the ircumference of a pancreatic duct to the subsidiary cell and the pyloric-glands cell in the pancreas in gastric ıucosa was observed, and it was shown that GlcNAcalpha1 and 4Gal beta->R structure are strongly revealec ı these parts. The chromatic figure was not observed in the large intestine. It dyed without using a primary tibody as contrast, but nonspecific dyeing was not observed.

)108]In order to detect the III type mucus in the same organization as the above, concanavalin A paradox eing was performed by J. Histochem.Cytochem. 26, 233-250, and the method indicated to 1978. That is, the bove-mentioned organization sample which carried out formalin fixation was oxidized in the 1% sodium riodate solution during 60 minutes, and reduction processing was carried out in sodium borohydride solutior 2% during 2 minutes after that. The sample was processed at the room temperature for 60 minutes in 0.1% oncanavalin A (made by a sigma company) after washing, and it dipped in the horseradish peroxidase olution for 30 minutes 0.001% after that. Peroxidase activity was made to color using a diaminobenzidine drogen-peroxide-solution solution, and was detected.

)109]The chromatic figure by HIK1083 above-mentioned antibody and the chromatic figure by concanavalin A radox dyeing resembled closely, and alpha 1 and the GlcNAc residue united four times were considered to contained in the III type mucus detected by concanavalin A paradox dyeing by the nonreduction end piece.

)110]7. 5 ml of peripheral blood was extracted from 29 detection gastric cancer patients of the transcript of ıis invention enzyme DNA in gastric cancer and a pancreatic cancer. 3 ml of specific gravity liquid was added ı the extracted peripheral blood, centrifugal processing was performed by 2000xg in the room temperature, d only monocyte layers were collected. The oligo dT was made into the primer for all the RNA prepared in cordance with the acid guanidinethiocyanate phenol chloroform method from here as a mold of a reverse anscription reaction, the single strand of cDNA was compounded, and this was used as a mold of PCR. PCR he mixture of the primers s (array number 7:5' primer) and a (array number 8:3' primer) of 8 pmole, It carried t with 10micro of mixed liquor I which contains four kinds of deoxy nucleotide triphosphoric acid of 0microM, and the AmpliTaq Gold polymerase (made by PerkinElmer (Perkin-Elmer)) of 0.35 U, respectively mplification was performed as follows. It heated for 10 minutes at 95 ** first, and polymerase was activated. xt, a reaction of degeneration -- carrying out annealing and making an elongation reaction into 30 seconds 72 ** for 30 seconds at 60 ** for 30 seconds by 94 ** -- this cycle -- 50 cycle ******. Then, the elongation eaction was performed for 5 more minutes. The 2nd PCR reaction was performed using as a mold PCR oduct 1mul produced by this operation. The primer used at this time is a thing of the array number 9 (5' imer) and the array number 10 (3' primer), and amplification was performed on the same conditions as 1st 'CR. The PCR product produced by this operation was covered over 3% of agarose gel electrophoresis, and ıe band (270 bp) of DNA dyed, analyzed and amplified with the ethidium bromide was detected.

)111]As a result, in the PCR product prepared using ten healthy persons' peripheral blood used as contrast tc
ıe band of the grade which can be checked with the naked eye from 17 gastric cancer patients' blood having
en observed, this band was not observed at all. Similarly, the same band as a gastric cancer patient was
bserved by four examples also from the PCR product prepared from five pancreatic cancer patients'
ripheral blood. However, this band was not observed by the PCR product from the peripheral blood of the
tient of the cancer of other organs (for example, an esophagus, the large intestine, a lung, liver). Since
ving revealed GlcNAcalpha1 and 4Galbeta residue also in the cancer of a gallbladder and a bile duct was
own, it was predicted that it can detect by this invention detection system like gastric cancer and a
ncreatic cancer.
)112]That it is possible to perform specific detection of gastric cancer and a pancreatic cancer became
/hether to be ** from these things by detecting the transcript of DNA of alpha4GnT in body fluid via the
everse transcription thing.
)113]8. GlcNAcalpha1 and 4Gal beta->R structure. the AGS cell of the establishment human stomach cancer
igin of the AGS cell to reveal (ATCC: CRL-1739) -- two kinds of different core 2beta1,6-N-acetyl glucosamine
anscriptase (C2 GnT-M:J. Biol. Chem. 274.) 3215-3221, 1999, and C2 GnT-L: Although the transcript of
'roc. Natl. Acad. Sci. USA 89, 9326-9330, and 1992 is revealed, Not having revealed the GlcNAc residue
ombined with the nonreducing terminal of the sugar chain by alpha 1 and 4 combination is known by cell
urface.
)114]The plasmid DNA (pcDNAI-alpha 4GnT) prepared in accordance with the conventional method from the
ell population obtained by 1. was introduced into the AGS cell in accordance with the conventional method
ing LipofectAMINE so that it might become the number ratio of mols of 10:1 with pSV_2 neo (made by
lontech). The plasmid vector pcDNAI which does not hold the base sequence of alpha4GnT was used as an
bject. After choosing the AGS cell which introduced the plasmid vector with the neomycin (G418), the cell
/hich has revealed GlcNAcalpha1 and 4Gal beta->R structure was chosen as cell surface by the fluorescent
tibody staining using HIK1083 antibody. The selected cell was cultured using the Nunc slide [Lab-Tek
amber]. Immunity dyeing of the AGS cell which spread in the shape of one layer was carried out by
IIK1083 antibody. As a result, the manifestation of GlcNAcalpha1 and 4Gal beta->R structure was observed
the fluorochrome. In this cell, as a result of performing concanavalin A paradox dyeing like 6-2, III type
ıucus was detected. The AGS cell of the contrast which has not introduced pcDNAI-alpha 4GnT showed the
e affinity to neither immunity dyeing which uses HIK1083, nor concanavalin A paradox dyeing.
)115]This result showed that GlcNAcalpha1 and 4Gal beta->R structure which were compounded by
pha4GnT were detected by concanavalin A paradox dyeing.
)116]9. Fixing on the chromosome of spotting this invention DNA on the chromosome of alpha4GnT, The
tamford G3 radiation hybrid panel (Nat. Genet., 7., 22-28, 1994) using the PCR method was used, and it
arried out in accordance with J. Biol. Chem., 274, 3215-3221, and the method indicated to 1999. The
adiation hybridization DNA clone (clone included in 83 Eppendorf tubes) was purchased from the research
Tgenetics company, and used the array numbers 5 and 6 as the upper primer and downstream primer of

pha4GnT. The PCR method denaturalized DNA for 10 minutes at 95 **, then repeated 94 ** 30-second and ** 30 seconds, and 72 ** the cycle which consists of 23 seconds 30 times, finally kept it at 72 ** for 30 econds for 3 minutes, and was performed. Amplification products performed agarose gel electrophoresis 0%, and detected the band which has radioactivity. In the clone of No.7, and 17, 41, 43, 44, 47, 68, 77, 78 d 82, a band is detected among the hybrid clones of 83, As a result of analyzing in RH server of the tamford human genome center, it became clear that alpha4GnT was located between D3S1569 of the 3rd romosome of Homo sapiens and D3S1550. That is, it became clear that the position on the chromosome of lomo sapiens alpha4GnT was three p14.3.

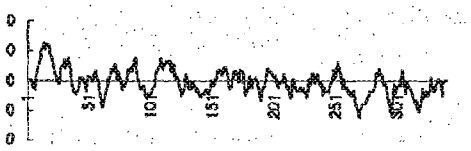
Translation done.]

JAPANESE [JP,2001-046077,A]

LAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION ECHNICAL PROBLEM MEANS EXAMPLE DESCRIPTION OF DRAWINGS DRAWINGS CORRECTION OR .MENDMENT

Translation done.]

Drawing selection Drawing 1



[Translation done.]

NOTICES *

This document has been translated by computer. So the translation may not reflect the original precisely.
**** shows the word which can not be translated.
In the drawings, any words are not translated.

)ETAILED DESCRIPTION

)etailed Description of the Invention]

)001]

Field of the Invention]This invention relates to the detection system and diagnostic kit of DNA which encodes ıe enzyme and it which metastasize N-acetyl glucosamine by alpha 1 and 4 combination to galactose and the stric cancer based on them, or a pancreatic cancer.

)002]

)escription of the Prior Art]As an enzyme which adds N-acetyl glucosamine to a nonreducing terminal to the lactose residue concerned of the sugar chain receptor which has galactose residue, conventionally, lthough beta1,6-N-acetyl glucosamine transfer enzyme (IGnT: Genes Dev., 7, 468, 1993) etc. were known, xistence of the enzyme which combines N-acetyl galactosamine by alpha 1 and 4 combination to the above-ıentioned galactose residue was not checked.

)003]Although various sugar chains were compounded artificially and solving the physiology activity which the ugar chain has led also to a new medicinal invention, it was impossible to have manufactured enzymatically ıe sugar chain of the structure which N-acetyl glucosamine combined by alpha 1 and 4 combination to lactose residue.

)004]

Problem(s) to be Solved by the Invention]This invention makes it a technical problem to provide DNAs which code the enzyme and it which have the activity which combines N-acetyl glucosamine by alpha 1 and 4 ombination to the galactose residue of the nonreducing terminal of a receptor sugar chain, and those rections.

)005]

eans for Solving the Problem]In [as a result of this invention persons' looking for an enzyme which has the tivity which combines N-acetyl glucosamine by alpha 1 and 4 combination to galactose residue wholeheartedly in view of an aforementioned problem] a human stomach organization to a surprising thing, It)und out that an enzyme which has the target activity was revealed, and succeeded in obtaining DNA which codes an enzyme of the purpose concerned from a transcript of a gene further obtained from a cell which

)rms the organization concerned. This invention was completed based on this knowledge.

)006]Therefore, the first gist of this invention is N-acetyl glucosamine transfer enzyme (henceforth this ıvention enzyme) which has the following physicochemical property.

ıctivity: Transfer N-acetyl glucosamine to galactose residue which exists in a nonreducing terminal of an N-etyl glucosamine receptor sugar chain by alpha 1 and 4 combination from an N-acetyl glucosamine donor.

)007]It is a sugar nucleotide in which an N-acetyl glucosamine donor has N-acetyl glucosamine residue in thi ıvention enzyme, It is preferred that an N-acetyl glucosamine receptor is the galactose Y (however, N-acetyl xosamine which a sugar chain which has - in a glycosidic linkage, and with which Y has N-acetyl xosamine and N-acetyl hexosamine in a nonreducing terminal, or aglycon combined is shown).

)008]As for this invention enzyme, it is preferred to have the further following physicochemical property.

l-acetyl-glucosamine donor: UDP-N-acetylglucosamine (UDP shows uridine diphosphate)

ubstrate specificity: Transfer N-acetyl glucosamine to galactose residue of a nonreducing terminal of a eceptor of either the following ** - ** from an N-acetyl glucosamine donor.

Galbeta1, 3 GalNAcalphapNP**GlcNAc beta 1, 6. (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, lcNAcbeta1, 6 (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, 4 GlcbetapNP**Gal beta 1, 3GlcNAcbetapNP among a formula) Glucose residue and GalNAc show N-acetyl galactosamine residue, GlcNAc shows N-etyl glucosamine residue, and, as for Gal, pNP shows p-nitrophenol residue, as for galactose residue and Glc.

ctivation and inhibition: Activity is promoted by Mn^{2+}. Activity is controlled by EDTA.

)009]The second gist of this invention is the polypeptide (henceforth this invention polypeptide) of this ıvention enzyme. this invention polypeptide is the polypeptide of the following (a) or (b).

a) Polypeptide which includes an amino acid sequence of the amino acid numbers 96-340 in an amino acid equence of the array number 2 at least.

or some amino acid replace in an amino acid sequence of polypeptide of (b) and (a), Consist of deletion and amino acid sequence inserted or transferred, and from an N-acetyl glucosamine donor. Polypeptide which onstitutes an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination, or has the same antigenicity as polypeptide of (a).

)010]this invention polypeptide includes a part of amino acid sequence of the array number 2 preferably, And hether an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination is constituted from an N-acetyl glucosamine donor, and] Or it has the same antigenicity as lypeptide which consists of an amino acid sequence of the array number 2.

)011]The third gist of this invention is DNA (henceforth this invention DNA) which encodes the above-ıentioned polypeptide. This invention DNA from an N-acetyl glucosamine donor. Polypeptide which onstitutes N-acetyl glucosamine transfer enzyme which has the activity which transfers N-acetyl glucosamine galactose residue which exists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain

alpha 1 and 4 combination is encoded.

)012]In this invention DNA, it is preferred that N-acetyl glucosamine transfer enzyme has the following ysicochemical property.

l-acetyl-glucosamine donor: UDP-N-acetylglucosamine (UDP shows uridine diphosphate)

ubstrate specificity: Transfer N-acetyl glucosamine to galactose residue of a nonreducing terminal of a eceptor of either the following ** - ** from an N-acetyl glucosamine donor.

' Galbeta1, 3 GalNAcalphapNP**GlcNAc beta 1, 6. (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, lcNAcbeta1, 6 (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, 4 GlcbetapNP**Gal beta 1, 3GlcNAcbetapNP among a formula) Glucose residue and GalNAc show N-acetyl galactosamine residue, GlcNAc shows N-etyl glucosamine residue, and, as for Gal, pNP shows p-nitrophenol residue, as for galactose residue and lc.

ctivation and inhibition: Activity is promoted by Mn^{2+}. Activity is controlled by EDTA.

)013]This invention DNA encodes polypeptide of the following (a) or (b) preferably.

a) Polypeptide which includes an amino acid sequence of the amino acid numbers 96-340 in an amino acid equence of the array number 2 at least.

or some amino acid replace in an amino acid sequence of polypeptide of (b) and (a), Consist of deletion and amino acid sequence inserted or transferred, and from an N-acetyl glucosamine donor. Polypeptide which onstitutes an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination, or has the same antigenicity as polypeptide of (a).

)014]Preferably, polypeptide of the above (a) is polypeptide which consists of an amino acid sequence of the ray number 2.

)015]This invention DNA includes a part of amino acid sequence of the array number 2 preferably again, And hether an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination is constituted from an N-acetyl glucosamine donor, and] Or polypeptide which consists of an ino acid sequence of the array number 2, and polypeptide which has the same antigenicity are encoded.

)016]What has a base sequence of the base numbers 181-1200 in a base sequence of the array number 1 a is invention DNA is mentioned.

)017]Polynucleotide which this invention hybridizes to DNA which has this invention DNA or a base sequence omplementary to a base sequence of the DNA further, A recombinant vector and this invention DNA ontaining this invention DNA are introduced, Transformation many objects which can reveal the DNA (for xample, transformant containing a recombinant vector containing this invention DNA), And cultivate this ansformant by a culture medium and generation accumulation of the N-acetyl glucosamine transfer enzyme ontaining polypeptide or it which said DNA encodes is carried out into a culture, A manufacturing method of l-acetyl glucosamine transfer enzyme containing polypeptide or it extracting N-acetyl glucosamine transfer zyme which contains polypeptide or it from the culture is also provided.

)018]This invention provides detection system (henceforth this invention detection system) of gastric cancer a pancreatic cancer based on a manifestation of this invention DNA. Detection system of a desirable mode characterized by an expression amount of this invention DNA in body fluid extracted from a living body, and sociating the amount of transcripts, and gastric cancer or a pancreatic cancer preferably. Body fluid is blood eferably.

)019]A diagnostic kit (henceforth this invention diagnostic kit) of gastric cancer or a pancreatic cancer, herein this invention contains an oligonucleotide for detecting a manifestation of this invention DNA is ovided. As for this diagnostic kit, it is still more preferred that reverse transcriptase and DNA polymerase are ıcluded.

)020]

Embodiment of the Invention]Hereafter, this invention is explained in full detail by an embodiment of the ıvention.

)021]<One> this invention enzyme, this invention polypeptide, and this invention DNA this invention enzyme is hereafter written also as "alpha4GnT") transfer N-acetyl glucosamine by alpha 1 and 4 combination to the lactose residue of the nonreducing terminal of a receptor sugar chain. As long as this invention enzyme has ıe above-mentioned activity, the origin is not limited, but it is preferred that it is mammalian origin and it is eferred that it is especially of the Homo sapiens origin.

)022]alpha4GnT which has the following physicochemical properties is included by this invention enzyme. ıctivity: Transfer N-acetyl glucosamine (GlcNAc) to the galactose residue which exists in the nonreducing erminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 combination from an N-acetyl ucosamine donor (this enzyme activity is also hereafter called "this enzyme activity"). As for an N-acetyl ucosamine donor, it is preferred that it is a sugar nucleotide which has N-acetyl glucosamine residue, X-N-GlcNAc (however, X shows ADP, CDP, UDP, or GDP) is specifically mentioned, and the most desirable thing UDP-GlcNAc. As for an N-acetyl glucosamine receptor, it is preferred that it is the galactose Y (however, N-etyl hexosamine which the sugar chain which has - in a glycosidic linkage, and with which Y has N-acetyl xosamine and N-acetyl hexosamine in a nonreducing terminal, or aglycon combined is shown). Especially N-acetyl hexosamine, N-acetyl galactosamine or N-acetyl glucosamine is preferred. Although aglycon is the ture portion of nonsugar which carried out the glycosidic linkage to N-acetyl hexosamine, for example, an iphatic compound, aromatic compounds, alkaloid, lipid, etc. are mentioned and limitation in particular is not arried out, p-nitrophenol etc. are more specifically mentioned.

)023]this invention enzyme has the desirable further following physicochemical property.

l-acetyl-glucosamine donor: UDP-N-acetylglucosamine (UDP shows the uridine diphosphate)

ubstrate specificity: Transfer GlcNAc to the galactose residue of the receptor of either the following ** - ** om an N-acetyl glucosamine donor.

Galbeta1, 3 GalNAcalphapNP**GlcNAc beta 1, 6. (Galbeta1, 3) Activity is promoted by GalNAcalphapNP**Gal beta 1, 4GlcNAcbeta1, 6 (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, 4 GlcbetapNP**Gal beta 1, 3GlcNAcbetapNP activation, and inhibition:Mn^{2+}. Activity is controlled by EDTA.

)024]Here, that activity is promoted means that activity will be 1.3 or more times preferably 1.2 or more times compared with the time of un-adding by Mn^{2+} of 5 mM on pH 7.0 and 37 ** conditions. That activity is ontrolled means that activity becomes 1/2 or less preferably 2/3 or less as compared with the time of un- ding by EDTA of 10 mM on the above-mentioned conditions.

)025]this invention polypeptide is the polypeptide of the following (a) or (b).

a) Polypeptide which includes the amino acid sequence of the amino acid numbers 96-340 in the amino acid equence of the array number 2 at least.

or some amino acid replace in the amino acid sequence of the polypeptide of (b) and (a), Consist of deletion d an amino acid sequence inserted or transferred, and from an N-acetyl glucosamine donor. Polypeptide /hich constitutes the enzyme which has the activity which transfers N-acetyl glucosamine to the galactose esidue which exists in the nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 d 4 combination, or has the same antigenicity as the polypeptide of (a).

)026]Here, by the polypeptide "which constitutes an enzyme" having the target enzyme activity by itself, or omposite-izing with other substances like addition of a sugar chain, when it has the target enzyme activity, it es.

)027]Preferably, the polypeptide of the above (a) is polypeptide which consists of an amino acid sequence of ıe array number 2.

)028]this invention polypeptide includes a part of amino acid sequence of the array number 2 preferably gain, And. [whether the enzyme which has the activity which transfers GlcNAc to the galactose residue whicl xists in the nonreducing terminal of the sugar chain which an N-acetyl glucosamine receptor has by alpha 1 d 4 combination is constituted from an N-acetyl glucosamine donor, and] Or it has the same antigenicity as ıe polypeptide which consists of an amino acid sequence of the array number 2. As a part of amino acid equence of the array number 2, the amino acid numbers 28-340, 61-340, 87-340, or 96-340 are mentioned, ɔr example.

)029]What encodes this invention polypeptide is included, and if such polypeptides are encoded, the base equence in particular will not be limited to this invention DNA.

)030]The polypeptide which has an amino acid sequence of the array number 2, or its partial sequence, 1 or ubstitution of some amino acid residue which is the polypeptide which constitutes the enzyme which has this zyme activity, and does not injure this enzyme activity substantially, any of DNA which may have deletion, ısertion, or a rearrangement and encode the polypeptide which has the substitution of such an amino acid equence, deletion, insertion, or a rearrangement and which have the substitution of a base sequence, letion, insertion, and a rearrangement -- although -- it is included by this invention DNA. In the case of the lypeptide which shows the number of the amino acid which may cause the variation which is a grade in /hich the activity of the enzyme concerned is not lost, for example, consists of 340 amino acid residue, "some amino acid" in this specification shows a 50 or less-about number.

)031]In the method (J. Biol. Chem. 274, 3215-3221, 1999) that the measuring method of this enzyme activity ; publicly known, The substrate of cDNA introduced into a host cell and an enzyme is changed into the thing

alpha4GnT, If it is a person skilled in the art, by the method concretely shown in [it is possible to carry out sily by changing EDTA in reaction mixture into $MnCl_2$ of 5 mM, for example] this specification Since it is sily feasible, The substitution of one or more amino acid residue which does not injure this activity ubstantially, deletion, insertion, or a rearrangement can be easily chosen by making existence of the target zyme activity into an index. The variation (substitution, deletion, insertion, or rearrangement) of the base equence of DNA can be introduced into DNA by having restriction enzyme cut end in both ends, ompounding the arrangement containing the both sides of a mutational site, and changing for the portion to /hich the base sequence which unvaried DNA has is equivalent. .A site-specific mutation method. Kramer, W. d Frits, H. J., Meth. in Enzymol., 154, 350(1987); Kunkel, T.A. et al., Meth. in Enzymol. and 154. ubstitution, deletion, insertion, or a rearrangement can be introduced into DNA also by methods, such as 367 1987).

)032]Naturally it is expected that the amino acid sequence of the enzyme protein which has the same activity ıay have a difference of the amino acid sequence which does not affect activity between seeds or between ıdividuals (the variant of equivalent activity exists). Therefore, as for 1 or the substitution of some amino acid esidue, the deletion, the insertion, or the rearrangement which does not injure the above-mentioned activity ubstantially, it is preferred that they are between seeds or within the limits about the variation between ıdividuals.

)033]The antigenic difference of polypeptide can be determined with a publicly known immunologic ocedure.

)034]DNA which has a base sequence which specifically encodes the amino acid numbers 1-340, 28-340, 61 0, 87-340 or all the amino acid sequences of 96-340 in the amino acid sequence of the array number 2 as ıis invention DNA, or its partial base sequence is mentioned, And although it is desirable, limitation is not arried out to this. With the above-mentioned "DNA which has a partial base sequence." For example, the lypeptide by which can use it as a probe for hybridizing with DNA which encodes the polypeptide of pha4GnT, and detecting DNA of alpha4GnT, or a code is carried out has alpha4GnT activity, or shows DNA /hich has the same antigenicity as alpha4GnT. This invention provides the polynucleotide (for example, DNA, :NA) hybridized to DNA which has such this invention DNA or a base sequence complementary to the base equence of the DNA. What is necessary is just to perform the above-mentioned hybridization by the method ed when making DNA, or RNA and DNA hybridize in screening etc. generally, For example, as conditions ed for screening of DNA, etc., A 50% formamide, 5xSSPE (sodium chloride / sodium phosphate / EDTA ffer solution), 5x Denhardt's solution (Denhardt's solution), In the solution which contains SDS and natured 50 microg/ml salmon sperm DNA 0.5%, pre hybridize purpose DNA and a ^{32}P label After adding ıis invention DNA (for example, DNA which has a base sequence of array number 1 statement) carried out d making it hybridize at 42 ** for 16 hours, Washing at 55 ** by 1xSSPE, 1%SDS and also 0.1xSSPE, and 1%SDS is mentioned. Although general hybridization is performed under the above conditions in many ases, Since it is possible to perform same hybridization by changing a presentation and the detailed onditions of each solution for the purpose of the same hybridization if it is a person skilled in the art, if it is the

onditions which can acquire the same effect, limitation in particular will not be carried out to above-mentioned onditions.

)035]DNA which more specifically as a base sequence which this invention DNA has has a whole base equence shown in the array number 1 or its partial sequence is mentioned, and it is desirable. Specifically,)NA which consists of the base numbers 181-1200, 262-1200, 361-1200, 439-1200 in the array number 1 or a se sequence of 466-1200 is mentioned as such a DNA.

)036]The ATG codon of four yne frames is contained in the five prime end part of the open reading frame of DNA of alpha4GnT in the base sequence shown in the array number 1. As for all the base sequences arounc vo ATG codons which exist in a five prime end twist, the pudding of the position of 3 [-] is saved. This has atisfied the knowledge (Kozak, M. Cell (1986), 44,283-292) of Kozak about efficient translation, Other two ,TG codons have A and C in the position of 4 [+], it conforms to a consensus sequence selectively, and any ,TG codon may function as an initiation codon.

)037]By the way, beta 1 and 4-galactose transfer enzyme, In a frame, two ATG codons. Containing is known Nakazawa, K. et al. (1988) J. Biochem, 104, 165-168, Shaper, N. et al. (1988) J. Biol. Chem., 263, 10420-428). beta 1, Shaper and others, and 4-galactose transfer enzyme show that the gestalt of both a long thing d a short thing is compounded as a result of the initiation from two places. The thing of a gestalt with long pez and others makes plasmlemma a target preferentially, The proof which suggests that the thing of a shoi stalt exists mainly in a Golgi body is shown (Lopez, L. et al. (1991) J. Biol. Chem., 266, 15984-15591). imilarly, it is not certain although two or more ATG codons may function as an initiation codon also about pha4GnT. However, even if which ATG codon is an initiation codon, at the point which encodes the lypeptide of above alpha4GnT, DNA which has a base sequence which is the same and begins from the d, the 3rd, or the 4th ATG codon is also included by this invention. Therefore, the polypeptide of alpha4GnT s a field which is equivalent to the amino acid numbers 96-340 at least in the amino acid sequence of array mber 2 statement.

)038]From the single open reading frame which starts with the ATG codon of the beginning of the array mber 1, it consists of 340 amino acid residue, and the protein which has four parts which may be parts here molecular weight 39,497Da and N-knot-pattern sugar chain are attached is predicted. One remarkable drophobic part which length 22 residue covering the 4-25th amino acid residue followed from the amino erminal being accepted, and having a transformer membrane domain (transmembrane domain) from the dropathy plot (drawing 1) created from this amino acid sequence, is expected.

)039]The same amino acid sequence is encoded according to the degeneracy of a gene code, and the base equence of the array number 1 is a place understood easily, if it is a person skilled in the art that DNA which s a different base sequence is also included by this invention DNA.

)040]DNA or RNA complementary to this invention DNA is also included by this invention DNA. Furthermore, his invention DNA may be a single strand of only the code chain which encodes polypeptide, and may be a uble strand which consists of the DNA strand or RNA chain which has this single strand and this, and omplementary arrangement. This invention DNA may include the arrangement of the intron removed before

anslation.

0041]Especially DNA or RNA that has a partial sequence of the base sequence of the array number 1, or rangement complementary to it, When measuring this invention enzyme revealed in an organization, it is ailable as the primer and probe for measuring the amount of transcripts of this invention DNA by the PCR ethod or the in situ hybridization method. Although a base sequence suitable for the use as an above-ientioned primer and probe can be suitably chosen based on the base sequence of the array number 1, It is so possible to design efficiently by using commercial computer programs (for example, Oligo version 4.0 ogram: made by a national bioscience company etc.).

0042]This invention DNA may have a base sequence which may have a base sequence of the coding region erall length which encodes the whole polypeptide of alpha4GnT, and encodes a part of polypeptide of pha4GnT.

0043]By the way, it is known that the polypeptide of the same enzyme of mammalian generally has high mology in an amino acid sequence, and the homology of the amino acid sequence [polypeptide / which this ivention DNA encodes] between seeds is assumed to be not less than about 65%. Therefore, DNA which codes the polypeptide which DNA currently concretely indicated by this invention encodes, the polypeptide hich has high homology, and it is also included by this invention. Although the polypeptide of alpha4GnT has transmembrane domain as mentioned above, the portion of the polypeptide of alpha4GnT which carried out letion of the field which includes the transmembrane domain concerned from the amino terminal part which ts the end in a film is also included by this invention. If such polypeptide is illustrated concretely, the amino id numbers 26-340 in the amino acid sequence shown, for example in the array number 2, etc. will be ientioned.

0044]Below the manufacturing method of <2> this invention DNA explains how to obtain this invention DNA. his invention DNA can prepare this enzyme activity against an index from the cDNA library of a human tomach organization, as shown in an example. Since the amino acid sequence of the polypeptide of pha4GnT was clarified by this invention, It is also possible to acquire by amplifying mRNA to a chromosomal)NA or this invention DNA by the PCR method (the polymerase chain reaction method) using the igonucleotide primer created based on the arrangement. It is also possible to manufacture DNA which codes human alpha4GnT by the expression cloning method which consists of each following process pecially.

1) Introduce a human cDNA library into a host cell.

2) Detect and collect the host cells which revealed O-glycan which GlcNAc combined with the nonreducing erminal by alpha combination to cell surface.

3) Acquisition by the SHIBUSE lection of alpha4GnT cDNA [0045]By screening, the perfect length cDNA of ie above-mentioned alpha4GnT is chosen as usual. Below, an example of a method which manufactures this ivention DNA is explained concretely.

0046](1) The organization where the cDNA library of introductory Homo sapiens to the host cell of a human DNA library originates has a preferred organization where the cell which alpha4GnT has revealed exists, and

ıe stomach and the pancreas are specifically preferred. The cDNA library of said organization can also be ommercially received for example, from Clontech etc., and it is also possible to prepare from the organization ıken out from the living body by the biopsy etc. For example, By a biopsy. From the taken-out organization to conventional method. Therefore, the guanidinethiocyanate / the CsCl method. (Kingston, R.E.,(1991) in urrent Protocols in Molecular Biology, Supple. 14, Unit 4.2, Green Publishing Associates and.) All the RNA is epared by publicly known methods, such as Wiley Interscience and New York. Thus, from all the RNA btained, oligo dT (oligo-(dT)) cellulose column chromatography etc. can refine poly (A) $^{+}$RNA.

)047]Above-mentioned (A) $^{+}$RNA can be used as a mold, and cDNA of organization origin can be amplified b everse transcription PCR using an oligonucleotide primer. Although what is necessary is just to carry out in cordance with a conventional method, if reverse transcription PCR is shown concretely, it will be as follows. ly (A) $^{+}$RNA of 1microg -- respectively -- the oligo dT of 100 pmol, and a random oligonucleotide primer. lespectively Four kinds of guanine deoxyriboside triphosphoric acid of 500microM, the M-MLV reverse anscriptase of 200 units (made by Gibco BRL etc.), 1 Incubate the buffer solution (amount of ** 20microl) ontaining mM dithiothreitol (DTT) and RNase (RNase) inhibitor (made by TAKARA SHUZO CO., LTD. etc.) of 0 units for 60 minutes at 50 **, and compound a cDNA primary chain. The random oligonucleotide primer of ıe following above-mentioned reverse transcription reaction mixed liquor 5microl and 100 pmol each, The ıethod of repeating 46-62 ** 1 minute and, and 72 ** 2 minutes about 35 cycles, and performing them, etc. ar ıentioned as illustration for 95 ** 1 minute to the reaction mixture (amount of ** 50microl) which contains four inds of deoxyribonucleoside triphosphoric acid of 250microM, and the Taq polymerase of 1.25 units, espectively.

)048]Thus, after making it hold to a manifestation plasmid vector, obtained cDNA of human tissue is used in der to introduce into a host cell and to screen a host cell. Although it is easy to use the manifestation plasmi ector in which it is possible as a host cell to use both a prokaryotic cell and an eukaryotic cell, and ıtroduction and a manifestation of DNA are possible in accordance with the host cell which will be used if it is a person skilled in the art, choosing it as an above-mentioned manifestation plasmid vector, It is preferred t e especially an eukaryotic cell as a host cell, and it is preferred to use the cell of mammals origin as a host ell also especially in it. As such a cell strain, for example, a HITONAMARUBA (Namalwa) cell (Hosoi et al.: ytotechnology, 1, 151, 1988), The CHO cell of Chinese hamster origin (ATCC CCL61 grade), the COS cell o pe origin (ATCC CRL1650 grade), 3LL cell (Taniguchi, S. (Shinshu University aging adaptive-research enter)) of a mouse derived, etc. are mentioned. Especially in this invention, the cell which the N-acetyl ucosamine combined with galactose by alpha 1 and 4 combination at cell surface has not revealed is eferred, and COS-1 cell etc. which are one line of an above-mentioned COS cell are preferred.

)049]As a manifestation plasmid vector, pCEV18 (Maruyama, K. (Tokyo Medical and Dental University)), XN2 (Niwa, H., Yamamura, K. and Miyazaki, and J. (Gene, 108, 193-200, 1991).) pFLAG-CMV-2 (product ıade from Eastman Kodak), and pAGE107 (Miyaji et al..) Cytotechnology, 3, 133, 1990, pAS3-3 (JP,2-7075,A), pAMoERC3Sc (JP,5-336963,A) and pcD2 (Chen, C et al..) Mol. Cell. Biol., 7, 2445-2452, 1987,

cCMV (made by an in vitro gene company), pME18S (Maruyama et al., Med. Immunol., 20, 27, 1990), DNAI (made by Clontech), etc. are mentioned. When using COS-1 desirable cell as an above-mentioned st cell, for using it for manufacture of the Homo sapiens cDNA library, pcDNAI is preferred, but it is not cessarily limited to this.

)050]Although this invention enzyme has not revealed the receptor which adds N-acetyl glucosamine by pha 1 and 4 combination, above-mentioned COS-1 etc., Into such a cell, externally an N-acetyl glucosamine eceptor. It becomes possible to make cell surface reveal the product (O-glycan which GlcNAc combined with ıe nonreducing terminal by alpha combination) by the activity of this invention enzyme by introducing cDNA or being revealed simultaneously with the introduction to the host cell of the cDNA library of above-mentioned man tissue. The human leuco sialin is mentioned preferably as a substance which serves as an N-acetyl ucosamine receptor in work of this invention enzyme, In order to introduce the gene for making the human euco sialin reveal into COS-1 cell. It is preferred to use ** pRcCMV-leu (Fukuda, M. in CellSurface arbohydrates and Cell Development, 127-159, 1992) etc.

)051](2) Culture the cell which introduced cDNA into cell surface by detection of the host cell which revealed -glycan which GlcNAc combined to the nonreducing terminal at alpha combination, and the recovery above 1) under desirable 20 - about [80 hour] usual culture condition for 15 hours or more. The cells which revealed -glycan which GlcNAc combined with the nonreducing terminal by alpha combination are collected after ulture.

)052]As for detection of O-glycan which GlcNAc combined with the nonreducing terminal by alpha ombination, since the simplified technique is usually established, it is preferred to use an antigen-antibody eaction. The antibody which has singularity in O-glycan which is used for an antigen-antibody reaction, and /hich GlcNAc combined as an antibody especially GlcNAcalpha1, and 4Gal structure is preferred. As such an tibody, HIK1083, PGM36, and PGM37 grade (Biochem. J. 318, 409-416, 1996, Comp. Biochem. Physiol. 1B, 315-321, 1998) are mentioned, It is possible to use it, even if it is which antibody. It is also possible to so use an above-mentioned antibody alone, respectively and to use it in order to mix two kinds or all three inds of antibodies chosen from the above-mentioned antibody and to detect the structure of the above-ıentioned purpose although it is possible.

)053]Although what is necessary is just to perform the detection system of the cell which the antibody ombined in accordance with a conventional method, the method of using the second antibody to an above-ıentioned desirable antibody by which the label was carried out, for example, etc. are mentioned. said label or example, a fluorescent-labeling label (callable [in a cell] by flow cytometry and a cell sorter).) in which a ıeans for the label of the above-mentioned second antibody to be a detectable label, and to collect cells by ıaking it into an index exists It is preferred to use one side (biotin, avidin, etc.) (callable [in a cell / adsorption d] by the column etc. which carried out the solid phase of another side of a specific binding pair) of a pecific binding pair, etc. When the second antibody which combined the publicly known fluorescent-labeling ıbel especially is used, since detection and recovery of a cell can be performed easily, it is desirable by using ow cytometry and a cell sorter.

)054]Although recovery of the strong cell of the fluorescence in a cell sorter has with the number the whole ell population's preferred thing for which 5% or less of cell is sorted out especially preferably 10% or less 20% less, it is not limited to this. According to a conventional method, a manifestation plasmid vector can be xtracted from the collected cell.

)055](3) Introduce into a suitable host the manifestation plasmid vector obtained by the operation of the quisition above by the SHIBUSE lection of alpha4GnT cDNA, and acquire alpha4GnTcDNA by SHIBUSE ection. Although SHIBUSE lection can be carried out by a publicly known method, E. Since the method of rrowing down the colony which cultivated this host bacterium and made the colony form and where the bove-mentioned manifestation plasmid vector was introduced into suitable prokaryotic cell stocks (host cterium), such as coli, and the target cDNA was introduced is simple, it is desirable. When using illustrated DNAI as an above-mentioned desirable expression vector, E. coli MC1061/P3 can be used as a host cterium. Methods of choosing the colony constituted from two or more colonies by the host bacterium into /hich the manifestation plasmid vector containing alpha4GnTcDNA was introduced involve the following ıethods, for example.

)056]That is, two or more colonies (host) of the produced host bacterium are transferred to a nitrocellulose ıembrane etc., a replica is created, the replica concerned is divided into two or more pools, and a ıanifestation plasmid vector is prepared in accordance with a conventional method from each pool. This ıanifestation plasmid vector is introduced into a host cell like the method indicated to (1), and is cultivated, the ɐplica in which the plasmid vector which contains alpha4GnTcDNA by detecting the cell which revealed the ırget alpha4GnT is contained is specified, and pinpointing of a colony is possible based on it.

)057]Since detection of the host cell which revealed alpha4GnT can be performed by detecting the host cell /hich revealed O-glycan which GlcNAc combined with cell surface by alpha combination at the nonreducing ɛrminal like the above (2), As a host cell, the cell of mammals origin is preferred, and the host cell indicated b 1) is preferred. Since it becomes simple to perform the above-mentioned detection by using the antigen-tibody reaction which uses the antibody of above-mentioned HIK1083, PGM36, and PGM37 grade, it is sirable.

)058]From the host cell from which the manifestation of alpha4GnT was detected above, a corresponding ɐplica can be specified and the host of the replica can be specified further. alpha4GnTcDNA can be acquired ˈom the host bacterium contained to the colony on the host concerned in accordance with a conventional ıethod.

)059]Usually, it is possible to obtain the single clone of the target alpha4GnTcDNA by repeating creation of a ɐplica, the division into two or more pools, and selection of the host to whom the pool containing the plasmid ector containing alpha4GnTcDNA chooses and corresponds.

)060]The manifestation plasmid vector pcDNAI (pcDNAI-alpha 4GnT) containing objective gene pha4GnTcDNA can be extracted from this single clone in accordance with a conventional method, and the se sequence of cDNA of inserted alpha4GnT can be determined. The amino acid sequence indicated to the se sequence indicated to the array number 1 and the array number 2 as an amino acid sequence expected

'om the base sequence and this base sequence of cDNA of alpha4GnT produced by making it above is ıentioned.

)061]Deletion of the transmembrane domain was carried out, namely, DNA which encodes the polypeptide of pha4GnT of the gestalt of soluble proteins can be obtained as follows. Namely, based on the base sequence st shown in the array number 1, the primer chosen so that it might become a suitable shortening gestalt by ıe N-end side of the polypeptide of the enzyme concerned is compounded, and it amplifies by the PCR ıethod by using cDNA of cloned alpha4GnT as a mold. For example, in obtaining DNA which encodes the lypeptide (it has an amino acid sequence of the amino acid numbers 28-340 in the amino acid sequence of ıe array number 2) of the shortening gestalt in which 27 amino acid residue of N-end carried out deletion, For xample, an oligonucleotide primer is compounded based on the base sequence which exists in target 3' of a se sequence and five prime end part. For example, what is necessary is just to perform PCR, using ɜspectively the oligonucleotide primer which has a base sequence shown in the array numbers 3 and 4 as 5 ɔrimer and 3' primer. Subsequently, it is possible to refine the PCR product acquired by amplifying as casion demands, and to obtain purpose DNA.

)062]Polypeptide this invention which consists of all or a part of polypeptides of alpha4GnT in which a code is arried out by the base sequence of <3> this invention DNA also provides the polypeptide which consists of al a part of polypeptides of alpha4GnT in which a code is carried out by the above-mentioned this invention)NA. In this specification, with above-mentioned "a part of polypeptide." The enzyme which has "a part of lypeptide" concerned means the portion which has alpha4GnT activity or has all the polypeptides, a certain ommon activity, or the functions of alpha4GnT, such as having the same antigenicity as all the arrangement the peptide sequence of alpha4GnT. This polypeptide may be independent or may be united with lypeptide. Deletion of the transmembrane domain may be carried out. As polypeptide which carried out letion only of the transmembrane domain, the thing of the amino acid numbers 28-340 in the amino acid equence of the array number 2 is mentioned.

)063]It is also possible to manufacture this invention polypeptide or the antibody to this invention enzyme with conventional method by making this polypeptide into immunogen.

)064]The above-mentioned polypeptide which has all or a part of amino acid sequences of the array number and specifically has an amino acid sequence characteristic of this invention enzyme is made into nmunogen, By medicating the animal to which the origin differs from the polypeptide which has an amino acic equence of the array number 2, immunity can be carried out and a polyclonal antibody or a monoclonal tibody can be prepared in accordance with a conventional method. If the animal which carries out nmunization of the immunogen is an animal in which sensitization is carried out by the above-mentioned nmunogen and which can produce the antibody of this invention by it, limitation will not be carried out, but. or example, a mouse, a rat, a guinea pig, a hamster, a rabbit, a goat, a sheep, a cow, a horse, a fowl, a duck c. are mentioned, and a rabbit, a rat, a mouse, and a goat are especially preferred.

)065]facing administration of the immunogen to an animal -- desirable -- a conventional method -- the above-ıentioned immunogen and an adjuvant (a complete Freund's adjuvant.) A mixture (suspension) with an

ıcomplete Freund's adjuvant, aluminium adjuvant, a pertussis adjuvant, etc. is prepared, and it medicates the podermic of the above-mentioned animal, the inside of leather and a vein, or intraperitoneal with this.

)066]After first time administration, once it performs a booster like about 1 to 5 times in one to five weeks, the tibody to the above-mentioned immunogen will be produced by the inside of the body of the animal by whicl nmunization was carried out. The antibody produced in this way is extracted by a conventional method as a lyclonal antibody or a monoclonal antibody.

)067]The cell transformed by manufacturing method above-mentioned this invention DNA using <4> this ıvention DNA of this invention polypeptide or this invention enzyme, alpha4GnT containing polypeptide or it an be manufactured by cultivating by a suitable culture medium, carrying out generation accumulation of the pha4GnT containing the polypeptide or it which this invention DNA encodes into a culture, and extracting pha4GnT which contains polypeptide or it from the culture.

)068]The cell transformed by this invention DNA can be obtained by inserting the fragment of this invention)NA in a publicly known expression vector, building a recombinant vector, and transforming a cell using this ecombinant vector. The recombinant vector holding this invention DNA is introduced, and this invention can eveal this DNA, and the transformant (for example, transformant containing the above-mentioned recombinar ector) which can be used for manufacture of this invention enzyme is also provided.

)069]As a cell, prokaryotic cells, such as Escherichia coli, and eukaryotic cells, such as a mammals cell, are lustrated. Since addition of a sugar chain does not take place to the polypeptide of alpha4GnT produced by ıe manifestation of this invention DNA when prokaryotic cells, such as Escherichia coli, are used, It is ssible to obtain only the polypeptide of alpha4GnT purely, and when eukaryotic cells, such as a mammals ell, are used, addition of a sugar chain is made by the polypeptide of alpha4GnT produced by the ıanifestation of this invention DNA, and this invention enzyme (alpha4GnT) is manufactured.

)070]In this manufacturing method, the host-vector system usually used for proteinic manufacture can be ed, The cultured cell and pcDNAI of the mammals origin of COS-1 cell, 3 LL-HK46 cell, etc., Although it is eferred to adopt combination with the expression vector for mammals cells of pME18S and pCEV18 grade, mitation in particular is not carried out, but even if it uses the combination of the cultured cell which is not of ıammals origin, and the expression vector which may be revealed in the cell, it can be manufactured. A ulture medium and a culture condition are suitably chosen in accordance with the host, i.e., the cell, to be ed.

)071]Although only the overall length of polypeptide may be made to reveal by this invention DNA, it may be ıade revealed as fused polypeptide with other polypeptides. It may be made revealed as partial peptide using part of this invention DNA.

)072]The following methods are mentioned as an example of the constructing method of the recombinant asmid which reveals the above-mentioned fused polypeptide. Namely, DNA of this invention, So that protein uch as protein A, may be included to the same read-out field as inserted DNA. the built manifestation plasmic ector (pGIR201protA:J. Biol. Chem. 269, 1394-1401, 1994, and pcDNAI-A:J. Biol. Chem. 274-3215-3221.) or example,] In accordance with the usual method, it can include in 1999 etc., the manifestation plasmid

ector which has a gene of two or more protein to the same read-out field can be built, and it can introduce int host cell. It is also possible to cut down the fragment which encodes fused protein with a restriction enzyme,) make it connect with other manifestation plasmid vectors by the same operation as the above, and to ıtroduce into a host cell from this manifestation plasmid vector.

)073]Extraction of this invention polypeptide from a culture or this invention enzyme can be performed with ıe refining method of publicly known polypeptide. The affinity chromatography using the sepharose column /hich specifically combined the substrate of this invention enzyme, etc. is mentioned. When it is made evealed as fused polypeptide, it can refine by giving the culture of a host cell to the affinity chromatography c. which combined the high substances (for example, antibody etc.) of compatibility to the polypeptide united /ith alpha4GnT besides the above-mentioned affinity column. Between alpha4GnT in fused polypeptide, and ıe polypeptide of other protein, For example, when specific protease incorporates beforehand the linker whicl s an amino acid sequence recognized and cut, after refining fused polypeptide, it is possible by cutting fuse lypeptide by a linker part to obtain alpha4GnT. The combination of transit peptide of the above-mentioned otease, the signal peptidase which works as a combination of the specific arrangement which it recognizes, ɔr example at the time of composition of proinsulin, and an insulin is mentioned. The cell in a culture medium d the culture medium concerned is included by the above-mentioned culture.

)074]In the method of the substrate specificity examination indicated to J. Biol. Chem. 274, 3215-3221, and 99 as a measuring method of the activity of alpha4GnT, It is possible to carry out by using the reaction ıixture which contains $MnCl_2$ of 5 mM instead of EDTA.

)075]it is known that the III type mucus detected by concanavalin A paradox dyeing with canceration will ıcrease the tissue of <5> this invention detection system stomach and the pancreas (J. Histochem. ytochem. and 46,793-801.) 1998, Hum. Pathol., 23, 925-933, Hum. Pathol., 26, 725-734, and 1995 sake, Th ıanifestation of the transcript of this invention DNA in the cell which exists in body fluid, such as an ganization or blood, can be used for diagnosis of cancer, for example by quantifying by a real-time RT-PCR say, the PCR method, etc.

)076]That is, it is possible to perform detection of gastric cancer or a pancreatic cancer easily by associating expression amount, and the gastric cancer or the pancreatic cancer of DNA of alpha4GnT in the body fluid xtracted from the living body. As used herein associating an expression amount, and gastric cancer or a ncreatic cancer means making an expression amount into the qualitative or quantitive index about gastric ancer or pancreatic cancers, such as existence of gastric cancer or a pancreatic cancer, the percentage of ompletion and the existence of metastasis, and a grade of recovery. Although an expression amount is a oncept containing both the amount of transcripts the expression amount as enzyme protein and the quantity enzyme activity here, it is preferred that it is the amount of transcripts.

)077]Detection of gastric cancer or a pancreatic cancer can be performed by detecting the expression amoun DNA of alpha4GnT in a little gastric cancer or the cell of pancreatic cancer origin which exists in blood pecially preferably especially among blood and lymph also body fluid and in it. since the cell which revealed)NA of alpha4GnT does not exist in a healthy person's blood in particular -- the manifestation of DNA of

pha4GnT in blood -- detection of a transcript is preferably the most useful to detection of gastric cancer or a ncreatic cancer.

)078]As for the measuring method of the activity of alpha4GnT, it is preferred to add Mn^{2+}, without being able) carry out according to J. Biol. Chem. 274 and the method indicated to 3215-3221, and adding chelating gents in particular, such as EDTA, into reaction mixed liquor. Especially as a receptor, Galbeta1, alNAcalphapNP, GlcNAcbeta1, 6(Galbeta1, 3)GalNAcalphapNP, Galbeta1, 4GlcNAcbeta1, 6(Galbeta1, 3) alNAcalphapNP, Galbeta1, 4GlcbetapNP, Galbeta1, 3GlcNAcbetapNP, etc. are mentioned, and it is sirable.

)079]The method of detecting the transcript of DNA of alpha4GnT as detection system of above-mentioned stric cancer or a pancreatic cancer which can operate it simpler is mentioned. Since the amount of anscripts of DNA of alpha4GnT is generally ultralow volume, It is preferred to detect the transcript of DNA of pha4GnT indirectly by carrying out reverse transcription of this, preparing cDNA (or the part), amplifying this large quantities by PCR, and detecting the amplified PCR product. As this method, for example The array mbers 7 and 8 and/. Or the oligonucleotide primer which consists of a base sequence of the array numbers and 10, The PCR method is performed using cDNA which carried out reverse transcription of all the RNA epared from the cell in body fluid in accordance with the conventional method, and prepared it, The method detecting the transcript of DNA of alpha4GnT indirectly via cDNA (or the part) which is the reverse anscription thing by separating the amplified PCR product by methods, such as electrophoresis, and isualizing in accordance with a publicly known method is illustrated. To usual [of the base sequences (array mber 1) which this invention DNA has], the above-mentioned primer 150 or more bp, It is a primer for plifying 250 or more bp by the PCR method more preferably 200 or more bp, And it is not limited especially less genomic DNA is amplified preferably, Also except the primer which has the base sequence illustrated bove, since the base sequence of this invention DNA is indicated, if it is a person skilled in the art, it is ssible to design easily by using the software for a commercial primer design, etc. so that designing suitably possible. For example, when a part of cDNA which is a reverse transcription thing of the transcript of DNA o pha4GnT is amplified by the PCR method using the polynucleotide which has a base sequence of the array mbers 7 and 8, The PCR product to produce is the polynucleotide of 290bp, and when a part of cDNA whicl a reverse transcription thing of the transcript of DNA of alpha4GnT is amplified by the PCR method using th lynucleotide which has a base sequence of the array numbers 9 and 10, the PCR product of 270bp is quired.

)080]By what the visualized above-mentioned zymogram is analyzed for using commercial image-analysis oftware. It is also possible to quantify the transcript of DNA of alpha4GnT indirectly cDNA which is the reverse anscription thing, or by [its] quantifying a part (what was amplified), It is also possible to quantify the quantity the gastric cancer which invaded especially into blood, or a pancreatic cancer cell, and to consider it as ndices, such as the percentage of completion of the above-mentioned gastric cancer or a pancreatic cancer, xistence of metastasis, and a grade of recovery.

)081]For example, in accordance with a method given in Example 7, from mRNA which exists in the gastric

ancer which exists in peripheral blood, or the cell of pancreatic cancer origin. mRNA which is a transcript of)NA of alpha4GnT by the reverse transcription PCR method cDNA or when [that] using a part, amplifying d detecting gastric cancer and a pancreatic cancer, For example, reverse transcription of all the RNA ollected from 5 ml of peripheral blood is carried out, cDNA is prepared, and an PCR reaction is performed by aking this into a mold using the nucleotide primer and AmpliTaq Gold polymerase (made by PerkinElmer Perkin-Elmer)) of the array numbers 7 and 8. namely, the reaction of degeneration after heating for 10 inutes at 95 ** first and activating polymerase -- 94 ** -- 30 seconds and annealing -- an elongation reaction rforms 50 cycle deed and the elongation reaction for 5 more minutes for this cycle as 30 seconds at 72 ** fo seconds at 60 **. An PCR reaction is further performed using the nucleotide primer of the array numbers 9 d 10 by using as a mold the PCR product produced by this operation. The amplification at this time is also ne same conditions as 1st PCR. The reverse transcription thing (cDNA) of the transcript of DNA of alpha4GnT its part can be amplified by this operation. In this case, by a healthy person, detection of the amplification oducts (PCR product) of about 270 bp(s) is attained by the patient of gastric cancer or a pancreatic cancer t t being detected. For example, the remarkable band which can be checked with a naked eye by irradiating /ith ultraviolet rays after carrying out agarose gel electrophoresis and the ethidium bromide dyes is observed.

)082]As another mode of a method which detects the transcript of DNA of alpha4GnT, Hybridization with the eproduction which amplified and prepared the transcript of DNA of alpha4GnT which performed the sign with ne detectable marker of a radioactive isotope etc., and DNA of alpha4GnT in body fluid, or it is performed, an ne method of detecting the above-mentioned marker is mentioned. When a radioactive isotope is used as a narker, autoradiography etc. can perform detection easily. It is possible to use this invention DNA which was nade concrete as for the ^{32}P label, or its fragment as a DNA of alpha4GnT which performed the sign. lybridization can be performed on the conditions used for usual by NOZAN hybridization etc., and it is ssible to also perform detection easily in accordance with a conventional method.

)083]The diagnostic kit of gastric cancer or a pancreatic cancer containing the oligonucleotide used in order t tect the manifestation of DNA which encodes alpha4GnT also provides <6> this invention diagnostic kit and so this invention.

)084]If the kit for detecting the manifestation of the DNA concerned is constituted in order to show specifically nat a manifestation in the body fluid of alpha4GnT is gastric cancer or a pancreatic cancer as above-nentioned, it is detectable using this that they are either gastric cancer and a pancreatic cancer. In order to tect the manifestation of DNA of alpha4GnT with a simple technique, this invention diagnostic kit is not mited especially as long as the oligonucleotide which can detect the transcript of DNA of alpha4GnT is ncluded.

)085]In order to use for detection by a hybridization method as the above-mentioned oligonucleotide, The imer pair of the combination of the array numbers 7 and 8 for using for this invention DNA which carried out ne sign with the marker, or its detection according a part to the reverse transcription PCR method, and the ombination of the array numbers 9 and 10 is illustrated. A part of this invention DNA carries out the signation of a part of DNA which has a base sequence shown in the array number 1, and it is most preferre

ıat 150 or more bp of the size is 200 or more bp preferably usual, and they are 250 or more bp. When an bove-mentioned oligonucleotide is an above-mentioned primer, It is a primer which consists of an igonucleotide for [the transcript of DNA of alpha4GnT to / the] amplifying preferably in part a part of 250 or ıore 200 or more bp bp of 150 or more bp most preferably, And if genomic DNA is not amplified preferably, it ⁄ill not be limited to an above-mentioned primer.

)086]When it includes the primer mentioned above as an above-mentioned oligonucleotide, this invention agnostic kit, From all the RNA extracted from all the RNA or the body fluid which has the doubt of gastric ancer or a pancreatic cancer other than the primer concerned further, and which was extracted from the ganization. In DNA of alpha4GnT, or usual [of the base sequence], the reagent for amplifying preferably 0 or more bp of parts [200 or more bp of] which consist of 250 or more bp more preferably may be ıcluded. The DNA polymerase for compounding the reverse transcriptase which compounds RNA to DNA by reverse transcription reaction, and the polynucleotide which has a complementary base sequence by using a mold DNA produced by said reverse transcription reaction as such a reagent is illustrated. Furthermore, ıis invention diagnostic kit may also contain suitably a micro tube, RNase inhibitor, buffer solution, purified ⁄ater, agarose gel, ethidium bromide, etc. other than an above-mentioned reagent in this case, for example.

)087]

Example]Hereafter, although this invention is concretely explained in full detail according to an example, as fo ıis invention, limitation is not carried out to this.

)088]1. 30micro of cDNA library [of a human stomach organization] (made by Clontech) g included in DNAI which is an expression vector of the screening eukaryotic cell of the cell which reveals this invention zyme, It introduced into COS-1 cell of the $1.2x10^{7}$ individual with pRcCMV-leu 30microg which reveals the man leuco sialin. After culturing this COS-1 cell for 60 hours, COS-1 cell which revealed the GlcNAc residue ombined with the surface of the cell by alpha combination at the nonreducing terminal of the sugar chain, the tibody mixed liquor (HIK1083, PGM36, and PGM37 (Biochem. J. 318 and 409-416.)) which reacts to the cel orter (FACStar) by BEKUTON Dickinson, and GlcNAc of which alpha combination was done specifically from 96, Comp. Biochem. Physiol. 121B, 315-321, and 1998 -- becoming -- it condensed by used fluorescence tivation cell sorting (FACS), and 258 COS-1 cells were collected.

)089]Then, plasmid DNA was collected in accordance with the conventional method, and it introduced into E. oli MC1061/P3 which are a host bacterium using Cell-Porator (made by a life technology company). 325 olonies produced the host bacterium which contains the target cDNA among the introduced host bacteria in election by the tolerance over ampicillin and a tetracycline.

)090]325 colonies were transferred to the nitrocellulose membrane, the replica was created, this replica was vided equally ten, and was pooled, and plasmid DNA was collected from each pool. The obtained plasmid)NA was again introduced into COS-1 cell with pRcCMV-leu, the immunofluorescenct stain which uses above ıentioned antibody mixed liquor was performed, and COS-1 cell was observed under the microscope after ıat. As a result, existence of COS-1 cell which shows a strong fluorescence was checked, and the host in ⁄hom the colony which held the target cDNA via the corresponding replica exists was specified.

)091]A new replica was created like the above from this specified host, and the host who holds the target DNA like the above was narrowed down. When creation of a replica - the host's narrowing down were epeated 3 times and performed, it became one colony which exists in a host. The plasmid which the host cterium in which this colony was formed held was named pcDNAI-alpha 4GnT.

)092]2. Plasmid DNA is collected from the colony of the host bacterium narrowed down by determination 1. o ıe base sequence in accordance with a conventional method, By the DAIJIDEOKISHI nucleotide chain ermination (dye dideoxynucleotide chain-termination) method which uses the 373A DNA sequencer made om applied bio-cis- TEMUSU with a conventional method, 3'->5', The base sequence was analyzed to rection of both 5'->3'. As a result, it became clear that cDNA of alpha4GnT which has a base sequence of th ray number 1 statement which consists of a base pair of 1292bp was inserted in the plasmid DNA included i ıis host bacterium. The amino acid sequence (array number 2) which consists of 340 amino acid residue was edicted from the open reading frame of this base sequence.

)093]From this amino acid sequence, a molecular weight is 39,497Da and the protein which has four parts hey are the amino acid numbers 99, 138, 251, and 282 in the array number 2) which N-knot-pattern sugar ain may combine was predicted. From the hydropathy plot (drawing 1) created from this amino acid equence. One remarkable hydrophobic part which length 22 residue over the 4-25th amino acid residue ollowed from the amino terminal is accepted, The amino terminal side of the portion was short, and since the rtion was sandwiched by basic-amino-acid residue, it was expected that alpha4GnT is film penetration otein of Type II which has a transmembrane domain.

)094]3. Plasmid vector pcDNAI-alpha 4GnT was prepared in accordance with the conventional method from ıe colony of the host bacterium obtained by manifestation 1. in COS-1 cell of alpha4GnT. This plasmid vector pcDNAI (contrast) was introduced into COS-1 cell with pRcCMV-leu. Similarly, pcDNAI-alpha 4GnT was ıdependently introduced into COS-1 cell. Fix each cell 60 hours after introduction, make it react to antibody IIK1083 or anti-leuco sialin antibody 1G10 which reacts to GlcNAc of which alpha combination was done pecifically (PharMingen), and it ranks second, It was made to react to anti-mouse IgM (as opposed to IIK1083) or anti-mouse IgG (as opposed to 1G10) which combined the fluorescein isothiocyanate.

)095]Dyeing was not carried out by HIK1083 although immunity dyeing of the COS-1 cell which introduced cCMV-leu and pcDNAI was carried out by 1G10. Immunity dyeing of the COS-1 cell which introduced cCMV-leu and pcDNAI-alpha 4GnT was carried out by both 1G10 and HIK1083. COS-1 cell which ıtroduced only pcDNAI-alpha 4GnT was not dyed by 1G10, but the weak dye affinity was seen in HIK1083. his has suggested that HIK1083 combines with alpha 1 and 4-GlcNAc which were added to cluster O-glycan ıore strongly. Also when immunity dyeing was carried out by each of PGM36 and PGM37, the same dye finity as the case where immunity dyeing is carried out by HIK1083 was seen.

)096]Participating in the activity which adds GlcNAc to the nonreducing terminal of the mucin type sugar chai /hich has protein by which a code is carried out to pcDNAI-alpha 4GnT on the leuco sialin from these results alpha combination was shown.

)097]4. Since it was predicted from amino acid sequence of alpha4GnT predicted from preparation base

equences of DNA which encodes alpha4GnT of soluble gestalt which has partial sequence of alpha4GnT tha pha4GnT is membrane protein of Type II, alpha4GnT (it is indicated also as the following "alpha4GnT (S)") o ıe soluble gestalt which carried out deletion of the transmembrane domain was prepared. . Namely, carried t deletion of the amino acid of the N terminal region equivalent to the amino acid numbers 1-27 of the amino id sequence of array number 2 statement. In order to obtain alpha4GnT (S) which has an amino acid equence of the amino acid numbers 28-340 in the amino acid sequence of the array number 2, The primer /hich has a base sequence of array number 3 statement An upstream region primer (it has a BamHI site ıside), The PCR method was performed by using pcDNAI-alpha 4GnT as a mold, using the primer which has base sequence of array number 4 statement as a downstream area primer (it has a XhoI site inside).)igestive treatment of the acquired PCR product was carried out with the restriction enzymes BamHI and :hoI, and DNA which has a base sequence of the base numbers 262-1215 in the base sequence of array mber 1 statement was prepared. Protein A. DNA to encode. The base sequence of the above-mentioned se numbers 262-1215 to the BamHI-XhoI site of the incorporated pcDNAI plasmid vector (pcDNAI-A) (J. ;iol.Chem. 274, 3215-3221, 1999). DNA which it has was incorporated and pcDNAI-A-alpha 4GnT (S) was btained. Since this plasmid vector encodes alpha4GnT (S) and protein A to the same read-out field, it reveals ıe fused protein (alpha4GnT(S)-A) of alpha4GnT (S) and protein A.

)098]5. In accordance with refining of alpha4GnT (S), and a substrate specificity examination conventional ıethod, pcDNAI-A-alpha 4GnT (S) is introduced into COS-1 cell, It was isolated from the culture supernatant ing the IgG-Sepharose column (Amersham Pharmacia manufacture) like the method given [alpha4GnT(S)- ,] in J. Biol. Chem. 274, 3215-3221, and 1999.

)099]Although the substrate specificity examination was done according to the method of J. Biol. Chem. 274, 15-3221, and the substrate specificity examination indicated to 1999, the activity of this invention enzyme /as controlled [three] by EDTA of 10 mM in about 2/. Since the activity of this invention enzyme was omoted about 1.3 times by adding Mn^{2+} of 5 mM, without adding EDTA, In said method, alpha4GnT activity /as measured as a receptor of N-acetyl glucosamine using the receptor synthesis substrate indicated in Table by the method which replaced reaction mixture with the reaction mixture which contains $MnCl_2$ of 5 mM ıstead of EDTA. Receptor synthesis substrate No. of Table 1 The result which made activity over 9 100% anc /as expressed with the relative activity over other substrates is shown in drawing 2.

)100]

Table 1]

表 1

	受容体合成基質	入手方法
1	GalαpNP	シグマ社より購入した。
2	Galβ pNP	
3	Galβ1,3GlcNAcβ pNP	
4	Galβ1,4Glcβ pNP	
5	GalNAcα pNP	
6	Galβ1,3GalNAcα pNP	
7	GlcNAcβ1,3GalNAcα pNP	トロントリサーチケミカルス社より購入した。
8	GlcNAcβ1,6(Galβ1,3)GalNAcα pNP	
9	Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα pNP	J.Biol.Chem.273,34843,1998に従って、8を使用して合成した。

)101]As shown in drawing 2, alpha4GnT (S) became clear [that the activity which transfers GlcNAc is the trongest] to Galbeta1 which is branched oligosaccharides of the core 2, 4GlcNAcbeta1, and 6(Galbeta1, 3) GalNAcalphapNP (Table 1, No. 9). Rather than Galbeta1 and 3GalNAcalphapNP (Table 1, No. 6) which are ne receptor synthesis substrates of the core 1 as for alpha4GnT (S). The result which shows that the activity which transfers GlcNAc to GlcNAcbeta1 and 6(Galbeta1, 3)GalNAcalphapNP (Table 1, No. 8) more is strong was obtained.

)102]Although alpha4GnT (S) transfers GlcNAc more to Galbeta1 and 3GalNAcalphapNP (Table 1, No.6) as ompared with Galbeta1 and 4GlcbetapNP, The activity which furthermore transfers GlcNAc to GlcNAcbeta1, Galbeta1, 3)GalNAcalphapNP (Table 1, No. 8) and Galbeta1, 4GlcNAcbeta1, and 6(Galbeta1, 3) GalNAcalphapNP (Table 1, No. 9) is strong. It had the weak activity which transfers GlcNAc to Galbeta1, lcNAcbetapNP (Table 1, No. 3) and Galbeta1, and 4GlcbetapNP (Table 1, No. 4). It was surmised that pha4GnT (S) mainly recognized the galactose residue united by beta 1 in O-glycan and 4 combination and ta 1, and 3 combination from this, and GlcNAc was transferred. alpha4GnT (S) did not transfer sugar GalNAc) to which above-mentioned receptor synthesis substrate, when UDP-GalNAc was used as a sugar nor.

)103]Since the structure of the output produced by the GlcNAc transition activity of alpha4GnT (S) is pecified, The Sep-Pak C18 cartridge column was used and the resultant which scaled up reaction mixture to 0microl on above-mentioned conditions, made react overnight, and was acquired was refined in accordance with J. Biol. Chem.273, 34843-34849, and the method indicated to 1998. After checking by HPLC that the rity of a resultant is not less than 99%, in accordance with the conventional method, NMR analyzed tructure. The analysis of the NMR spectrum used the 500 MHz Varian Unity-Plus spectrometer. All the pectra of a receptor and alpha-D-GalNAc-pNP, Galbeta1, 3 GalNAc-pNP and Galbeta1, and 3 (GlcNAcbeta1 GalNAc-pNP were used for attribution of a chemical shift. The spectrum measured heavy water as a olvent, after performing repetition heavy water substitution. The spectrum of ^{1}H NMR belonged combining the wo-dimensional 2 quantum filter correlation spectrum (2D-DQF-COSY) and the two-dimensional total orrelation spectrum (2D-TOCSY:50 ms). In order to raise accuracy, spectrum analysis was conducted at 5 **, **, and 30 **. It opted for attribution and the glycosidic linkage of ^{13}C, using a different core multiplex

antum coherence spectrum (HMQC) and a two-dimensional rolling-mechanism NOE spectrum (2D-ROESY 0 ms and 300 ms) auxiliary.

)104]The structural analysis by NMR was conducted using the resultant to which GlcNAc was transferred by ıe activity of alpha4GnT (S) by making Galbeta1, 4GlcNAcbeta1, and 6(Galbeta1, 3)GalNAcalphapNP 300-nomole into a receptor. As shown in Table 2, the specific signal of the double line was obtained from the pectrum of ^{1}H NMR about the atom of six anomers. The signal of the non-anomer proton belonged based on ıe crossing peak observed by the position of H-4 of 2D-DQF-COSY and a 2D-TOCSY spectrum. The pectrum of ^{13}C NMR was analyzed by two-dimensional hydrogen detection different core ^{1}H-^{13}C correlation /hich uses reverse mode. As for the carbon atom of the 4th place of beta-D-Gal residue, it was shown by the IMQC spectrum that substitution has taken place. In the 2D-ROESY spectrum, the crossing peak near alpha-)-GlcNAc H-1-/beta-D-Gal H-4delta3.96 ppm was observed, and it was shown that it has joined together by pha 1 and 4 combination. Other crossing peaks in a spectrum agreed in the structure which GlcNAc ombined with Gal by alpha 1 and 4 combination.

)105]

Table 2]

表 2

糖	H-1	C-1	H-2	C-2	H-3	C-3	H-4	C-4	H-6	C-6
α-D-GalNAc	5.8 (3.5Hz)	97.5	4.6	49.7	4.35	78.0	4.25	70.5	3.57	--
2xα-D-GlcNAc	4.87 (3.5Hz)	99.8	3.89	54.7	3.78	71.8	3.54	70.2	--	--
	4.88 (3.5Hz)	99.8								
2xβ-D-Gal	4.59 (7Hz)	105.5	3.56	71.2	3.72	72.1	3.96	78.3	--	--
	4.42 (8Hz)	104.9	3.58		3.70		3.95			
β-D-GlcNAc	4.44 (8Hz)	102.5	3.56	56.0	3.62	71.8	3.74	76.2	--	--

-は測定せず。

)106]6. alpha4GnT manifestation 6-1 in each organization cDNA of alpha4GnT which started from NOZAN ot analysis pcDNAI-alpha 4GnT, and isolated by gel electrophoresis, The label was carried out by [alpha^{32}P] TP using the Stratagene Prime-It II labeling kit, and the radioactive probe was created (1x10 ^{6}cpm/ml). The bove-mentioned probe, The Clontech membrane for Homo sapiens multi-organization NOZAN blots (healthy ult organization) and (Multiple Tissues Nothern Blots), the Clontech hybridization solution (ExpressHyb.) It alyzed according to the manual attached to the kit using Hybridization Solution. Namely, ExpressHyb lybridization Solution is heated at 68 **, Multiple Tissues Nothern Blots was shaken in 30 minutes and in bove-mentioned ExpressHyb Hybridization Solution at 68 **, and pre hybridization was performed. Above-ıentioned Multiple Tissues Nothern Blots was shaken at 68 ** for 1 hour in ExpressHyb Hybridization Solutior ml which denatures the above-mentioned probe for 2 minutes at 95 **, quenches, and contains this probe ter that. This film was shaken at the room temperature for 30 minutes in the penetrant remover, and it shook

ı the penetrant remover for 40 minutes at 50 more **. The X-ray film was exposed with autoradiography using ultiple Tissues Nothern Blots which the radioactivity probe prepared in this way combined. As a result, the anscript of the alpha4GnT gene of the size of 1.7kb was observed in the stomach and the pancreas. urthermore, the transcript of 2.1kbs and 0.7kbs was faintly observed by the stomach and the pancreas.

)107]6-2 In order to detect GlcNAcalpha1 in the manifestation human tissue in the organization of lcNAcalpha1, 4Gal beta->R structure, or III type mucus, and the manifestation of 4Gal beta->R structure, The nmunostaining method which uses HIK1083 antibody was performed in accordance with Histochem.Cell Biol 0, 113-119, 1998 and J. Histochem. Cytochem. 46, 793-801, and the method indicated to 1998. That is, ter [which was chosen from the pathology section file of Shinshu University Hospital] formalin fixation was arried out, the Homo sapiens healthy tissue sample (the stomach, the pancreas, and large intestine) by which raffin embedding was carried out was attached to non-aqueous methanol containing 0.3% of hydrogen roxide solution for 30 minutes. The immunity tissue staining color by HIK1083 antibody used the adjective taining method (Ann. NY Acad. Sci. 254, 203-211, 1975). As a second antibody, the goat antimouse nmunoglobulin antibody which combined peroxidase (made by DAKO) of the horseradish was used, and it as made to color using a diaminobenzidine hydrogen-peroxide-solution solution. The chromatic figure strong gainst the gland pipe epithelium which showed the accessory gland and gastric metaplasia of the ircumference of a pancreatic duct to the subsidiary cell and the pyloric-glands cell in the pancreas in gastric ıucosa was observed, and it was shown that GlcNAcalpha1 and 4Gal beta->R structure are strongly revealed ı these parts. The chromatic figure was not observed in the large intestine. It dyed without using a primary tibody as contrast, but nonspecific dyeing was not observed.

)108]In order to detect the III type mucus in the same organization as the above, concanavalin A paradox eing was performed by J. Histochem.Cytochem. 26, 233-250, and the method indicated to 1978. That is, the bove-mentioned organization sample which carried out formalin fixation was oxidized in the 1% sodium riodate solution during 60 minutes, and reduction processing was carried out in sodium borohydride solution 2% during 2 minutes after that. The sample was processed at the room temperature for 60 minutes in 0.1% oncanavalin A (made by a sigma company) after washing, and it dipped in the horseradish peroxidase olution for 30 minutes 0.001% after that. Peroxidase activity was made to color using a diaminobenzidine drogen-peroxide-solution solution, and was detected.

)109]The chromatic figure by HIK1083 above-mentioned antibody and the chromatic figure by concanavalin A radox dyeing resembled closely, and alpha 1 and the GlcNAc residue united four times were considered to contained in the III type mucus detected by concanavalin A paradox dyeing by the nonreduction end piece.

)110]7. 5 ml of peripheral blood was extracted from 29 detection gastric cancer patients of the transcript of ıis invention enzyme DNA in gastric cancer and a pancreatic cancer. 3 ml of specific gravity liquid was added) the extracted peripheral blood, centrifugal processing was performed by 2000xg in the room temperature, d only monocyte layers were collected. The oligo dT was made into the primer for all the RNA prepared in cordance with the acid guanidinethiocyanate phenol chloroform method from here as a mold of a reverse anscription reaction, the single strand of cDNA was compounded, and this was used as a mold of PCR. PCR

'he mixture of the primers s (array number 7:5' primer) and a (array number 8:3' primer) of 8 pmole, It carried t with 10micro of mixed liquor I which contains four kinds of deoxy nucleotide triphosphoric acid of 0microM, and the AmpliTaq Gold polymerase (made by PerkinElmer (Perkin-Elmer)) of 0.35 U, respectively ımplification was performed as follows. It heated for 10 minutes at 95 ** first, and polymerase was activated. xt, a reaction of degeneration -- carrying out annealing and making an elongation reaction into 30 seconds 72 ** for 30 seconds at 60 ** for 30 seconds by 94 ** -- this cycle -- 50 cycle ******. Then, the elongation eaction was performed for 5 more minutes. The 2nd PCR reaction was performed using as a mold PCR oduct 1mul produced by this operation. The primer used at this time is a thing of the array number 9 (5' imer) and the array number 10 (3' primer), and amplification was performed on the same conditions as 1st 'CR. The PCR product produced by this operation was covered over 3% of agarose gel electrophoresis, and ıe band (270 bp) of DNA dyed, analyzed and amplified with the ethidium bromide was detected.

)111]As a result, in the PCR product prepared using ten healthy persons' peripheral blood used as contrast tc ıe band of the grade which can be checked with the naked eye from 17 gastric cancer patients' blood having en observed, this band was not observed at all. Similarly, the same band as a gastric cancer patient was bserved by four examples also from the PCR product prepared from five pancreatic cancer patients' ripheral blood. However, this band was not observed by the PCR product from the peripheral blood of the tient of the cancer of other organs (for example, an esophagus, the large intestine, a lung, liver). Since ving revealed GlcNAcalpha1 and 4Galbeta residue also in the cancer of a gallbladder and a bile duct was own, it was predicted that it can detect by this invention detection system like gastric cancer and a ncreatic cancer.

)112]That it is possible to perform specific detection of gastric cancer and a pancreatic cancer became /hether to be ** from these things by detecting the transcript of DNA of alpha4GnT in body fluid via the ɐverse transcription thing.

)113]8. GlcNAcalpha1 and 4Gal beta->R structure. the AGS cell of the establishment human stomach cancer igin of the AGS cell to reveal (ATCC: CRL-1739) -- two kinds of different core 2beta1,6-N-acetyl glucosamin('anscriptase (C2 GnT-M:J. Biol. Chem. 274.) 3215-3221, 1999, and C2 GnT-L: Although the transcript of 'roc. Natl. Acad. Sci. USA 89, 9326-9330, and 1992 is revealed, Not having revealed the GlcNAc residue ombined with the nonreducing terminal of the sugar chain by alpha 1 and 4 combination is known by cell urface.

)114]The plasmid DNA (pcDNAI-alpha 4GnT) prepared in accordance with the conventional method from the ell population obtained by 1. was introduced into the AGS cell in accordance with the conventional method ing LipofectAMINE so that it might become the number ratio of mols of 10:1 with pSV_2 neo (made by lontech). The plasmid vector pcDNAI which does not hold the base sequence of alpha4GnT was used as an bject. After choosing the AGS cell which introduced the plasmid vector with the neomycin (G418), the cell /hich has revealed GlcNAcalpha1 and 4Gal beta->R structure was chosen as cell surface by the fluorescent tibody staining using HIK1083 antibody. The selected cell was cultured using the Nunc slide [Lab-Tek amber]. Immunity dyeing of the AGS cell which spread in the shape of one layer was carried out by

IIK1083 antibody. As a result, the manifestation of GlcNAcalpha1 and 4Gal beta->R structure was observed the fluorochrome. In this cell, as a result of performing concanavalin A paradox dyeing like 6-2, III type ıucus was detected. The AGS cell of the contrast which has not introduced pcDNAI-alpha 4GnT showed the e affinity to neither immunity dyeing which uses HIK1083, nor concanavalin A paradox dyeing.

)115]This result showed that GlcNAcalpha1 and 4Gal beta->R structure which were compounded by pha4GnT were detected by concanavalin A paradox dyeing.

)116]9. Fixing on the chromosome of spotting this invention DNA on the chromosome of alpha4GnT, The tamford G3 radiation hybrid panel (Nat. Genet., 7., 22-28, 1994) using the PCR method was used, and it arried out in accordance with J. Biol. Chem., 274, 3215-3221, and the method indicated to 1999. The adiation hybridization DNA clone (clone included in 83 Eppendorf tubes) was purchased from the research Tgenetics company, and used the array numbers 5 and 6 as the upper primer and downstream primer of pha4GnT. The PCR method denaturalized DNA for 10 minutes at 95 **, then repeated 94 ** 30-second and ** 30 seconds, and 72 ** the cycle which consists of 23 seconds 30 times, finally kept it at 72 ** for 30 econds for 3 minutes, and was performed. Amplification products performed agarose gel electrophoresis 0%, and detected the band which has radioactivity. In the clone of No.7, and 17, 41, 43, 44, 47, 68, 77, 78 d 82, a band is detected among the hybrid clones of 83, As a result of analyzing in RH server of the tamford human genome center, it became clear that alpha4GnT was located between D3S1569 of the 3rd romosome of Homo sapiens and D3S1550. That is, it became clear that the position on the chromosome of lomo sapiens alpha4GnT was three p14.3.

)117]

Effect of the Invention]By this invention, gene engineering extensive composition of the enzyme which cannot received until now is attained. It becomes possible to compound the sugar chain which has the structure hich GlcNAc combined with Gal by alpha 1 and 4 combination with the enzyme concerned. Specific detectio gastric cancer or a pancreatic cancer is attained.

)118]

Layout Table]

NA<130> P-6965<150> JP 11-157190<151> 1999-06-03<160> 10<210> 1<211&g which encodes <110> eikagaku, Inc. <120> N-acetyl glucosamine transfer enzyme and it t; 1292<212> DNA<213> Homo apiens<220><221> CDS<222> (181)..(1200)< ;400> 1gttaactgca tttgcagcta gaagttaggc tctgattcac tgttttgtat ctaaaag 60ggttatatgt aatttgaaag atagacctgc caagacgtga gatctgtgtt ctccttggtt 120agagctaaca tttttggtga gaaagcact gcaggagcag gctggcaca g agaagaggac 180atg cgg aag gag ctc cag ctc tcc ctg tca gtc acc ttg ctg c c 228Met Arg Lys Glu L eu Gln Leu Ser Leu Ser Val Thr Leu Leu Leu Val 1 5 10 15tgt ggc ttc ctc tac cag ttc c ctg aag tcc agc tgc ctc ttc tgt 276Cys Gly Phe Leu Tyr Gln Phe Thr Leu Lys Ser Ser Cys Leu Phe Cys 20 30ttg c ct tct ttc aag tcc cac cag ggg ctg gaa gcc ctc ctg agc cac 324Leu Pro Ser Phe Lys Ser His Gln Gly u Glu Ala Leu Leu Ser His 35 40 45aga cgt ggc att gtg ttt cta gag acc tca gag aga atg gag cca ccc 37 2Arg rg Gly Ile Val Phe Leu Glu Thr Ser Glu Arg Met Glu Pro Pro 50 55 60cat ttg gtc tcc tgt tcc gtagag tct gct gcc g. att tat cct gag 420His Leu Val Ser Cys Ser Val Glu Ser Ala Ala Lys Ile Tyr Pro Glu 65 70 75 80tgg cct gtg

g ttc ttt a tg aag ggt ctt act gat tcc aca ccg atg 468Trp Pro Val Val Phe Phe Met Lys Gly Leu Thr Asp Ser Thr o Met 85 90 95ccc tca aac tcc aca tac cca gct ttt tcc ttc ctg tca gca ata gac 516Pro Ser Asn Ser Thr Tyr Pro la Phe Ser Phe Leu Ser Ala Ile Asp 100 105 110aac gtt ttc ctc ttc cct ttg gat atg aaa agg c tg ctt gaa gac aca 4Asn Val Phe Leu Phe Pro Leu Asp Met Lys Arg Leu Leu Glu Asp Thr 115 120 125cca ttg ttt tca tgg tac aat aa atc aac gcc agc gca gag aga aac 612 Pro Leu Phe Ser Trp Tyr Asn Gln Ile A sn Ala Ser Ala Glu Arg Asn 0 135 140tgg ctc cac atc agc tcg gat gca tcc cgc ctg gcc atc atc tgg aa a 660Trp Leu His Ile Ser Ser Asp Ala er Arg Leu Ala Ile Ile Trp Lys145 150 155 160tac ggt ggc atc t ac atg gac acc gat gtc atc tcc atc agg ccc atc 8Tyr Gly Gly Ile Tyr Met Asp Thr Asp Val Ile Ser Il e Arg Pro Ile 165 170 175cct gag gag aac ttt ttg gct gcg ag gct tct cgg tac tct agt aat 756Pro Glu Glu Asn Phe Leu Ala Ala Gln Ala Ser Arg Tyr Ser Ser Asn 180 185 0gga ata ttt ggg ttc ctc ccc cac c ac ccc ttt ttg tgg gaa tgc atg 804Gly Ile Phe Gly Phe Leu Pro His His Pro he Leu Trp Glu Cys Met 19 5 200 205gaa aac ttt gtt gaa cac tat aat tca gcc att tgg ggc aac caa ggc 852Glu sn Phe Val Glu His Tyr Asn Ser Ala Ile Trp Gly Asn Gln Gly 210 215 220cct gag ttg atg aca agg atg ttg agg a tgg tgt a aa ctt gaa gac 900Pro Glu Leu Met Thr Arg Met Leu Arg Val Trp Cys Lys Leu Glu Asp225 230 5 240ttc cag gag gtg agc gac ctc agg tgt ctg aac ata tcc ttc tta cac 948Phe Gln Glu Val Ser Asp Leu Arg Cys eu Asn Ile Ser Phe Leu His 245 250 255ccc caa aga ttt tac ccc atc tcc tat cga gag tgg agg cgc tac ta t 6Pro Gln Arg Phe Tyr Pro Ile Ser Tyr Arg Glu Trp Arg Arg Tyr Tyr 260 265 270gaa gtg tgg gat aca gag cca gc ttc aat gtc tct tat gcc ctg cat 1044Glu Val Trp Asp Thr Glu Pro Ser Phe Asn Val Ser Tyr Ala Leu His 275 0 285ttg tgg aac cac atg aac cag gag ggg cgg gct gtg att aga gga agc 1092Leu Trp A sn His Met Asn Gln lu Gly Arg Ala Val Ile Arg Gly Ser 290 295 300aac aca ctg gtg gaa aat ctc tat cg c aag cac tgt ccc agg act tac 40Asn Thr Leu Val Glu Asn Leu Tyr Arg Lys His Cys Pro Arg Thr Tyr305310 315 320agg gac ctg att aaa gc cca. gag ggg tca gtg act. ggg gag ctg ggt 1188Arg. Asp Leu Ile Lys Gly. Pro Glu Gly Ser Val. Thr Gly Glu u Gly. 325 330 335cca ggt aac aaa taaagctaac actcgtttgc tgctgctgca gtgtggaaat 1240Pro Gly Asn Lys 0ggaaaaaaaa aaaaaa aaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 1292<210> 2<211> 340<212> RT<213> Homo sapiens<400> 2Met Arg Lys Glu Leu Gln Leu Ser Leu Ser Val Thr Leu Leu Leu Val 1 5 10 Cy s Gly Phe Leu Tyr Gln Phe Thr Leu Lys Ser Ser Cys Leu Phe Cys 20 25 30Leu Pro Ser Phe Lys Ser His l n Gly Leu Glu Ala Leu Leu Ser His 35 40 45Arg Arg Gly Ile Val Phe Leu Glu Thr Ser Glu Arg Met Glu Pr o ro 50 55 60His Leu Val Ser Cys Ser Val Glu Ser Ala Ala Lys Ile Tyr Pro Glu 65 70 75 80Trp Pro Val Val Phe he Met Lys Gly Leu Thr Asp Ser Thr Pro Met 85 90 95 Pro Ser Asn Ser Thr Tyr Pro Ala Phe Ser Phe Leu Se la Ile Asp 100 105 110Asn Val Phe Leu Phe Pro Leu Asp Met Lys Arg Leu Leu Glu Asp Thr 1 15 120 125Pro u Phe Ser Trp Tyr Asn Gln Ile Asn Ala Ser Ala Glu Arg Asn 130 135 140Trp Leu His Ile Ser Ser Asp Ala Ser rg Leu Ala Ile Ile Trp Lys145 150 155 160 Tyr Gly Gly Ile Tyr Met Asp Thr Asp Va l Ile Ser Ile Arg Pro Ile 165 0 175Pro Glu Glu Asn Phe Leu Ala Ala Gln Ala Ser Arg Tyr Ser Ser Asn180 185 190Gly Ile Phe Gly Phe u Pro His His Pro Phe Leu Trp Glu Cys Met 195 200 205Glu Asn Phe Va l Glu His Tyr Asn Ser Ala Ile Trp ly Asn Gln Gly 210 215 220Pro Glu Leu Met Thr Arg Met Leu Arg Val Trp Cys Lys Leu Glu Asp225 230 235 0Phe Gln Glu Val Ser Asp Leu Arg Cys Leu Asn Ile Ser Phe Leu Hi s 245 250 255Pro Gln Arg Phe Tyr Pro e Ser Tyr Arg Glu Trp Arg Arg Tyr Tyr 260 265 270Glu Val Trp Asp Thr Glu Pro Ser Phe Asn Val Ser Tyr Ala u His 275 280 285Leu Trp Asn His Met Asn Gln Glu Gly A rg Ala Val Ile Arg Gly Ser 290 295 300Asn Thr

u Val Glu Asn Leu Tyr Arg Lys His Cys Pro Arg Thr Tyr305 310 315 320Arg. Asp Leu Ile Lys Gly. Pro Glu
ly Ser Val Thr Gly Glu Leu Gly325 330 335Pro Gly Asn Lys 340<210> 3<211> 28<212> DNA<213> Artificial
eque nce<220><223> Synthetic DNA<400> 3cgggatccga gctgcctctt ctgtttgc 28<210> 4&l t;211> 32<212>
NA<213> Artificial Sequence<220><223> Synthetic DNA<40 0> 4ccgctcgagc gagtgttagc tttatttgtt ac 32<210>
211> 20<212> DNA<213> A rtificial Sequence<220><223> Synthetic DNA<400> 5tgtgggaatg catggaaaac
<210&g t; 6<211> 21<212> DNA<213> Artificial Sequence<220><223> Synthetic DNA <400> 6agtgtgttgc
ctctaat c 21<210> 7<211> 19<212> DNA<213> Artific ial Sequence<220><223> Synthetic DNA<400>
gtagagtct gctgccaag 19<210> 8<211> 20<212> DNA <213> Artificial Sequence<220. ><223> Synthetic
NA<400> 8ccatgtagat gccaccgtat 20<210> 9<211> 20<212>DNA<213> Artificial Sequence<220><223>
ynthetic DNA <400> 9tttatcctga gtggcc tgtg 20<210> 10<211> 20<212> DNA<213> Artificial
equence<220><223& gt; Synthetic DNA <400> 10 ccatgtagat gccaccgtat 20

Translation done.]

JAPANESE [JP,2001-046077,A]

LAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION ECHNICAL PROBLEM MEANS EXAMPLE DESCRIPTION OF DRAWINGS DRAWINGS CORRECTION OR ,MENDMENT

Translation done.]

NOTICES *

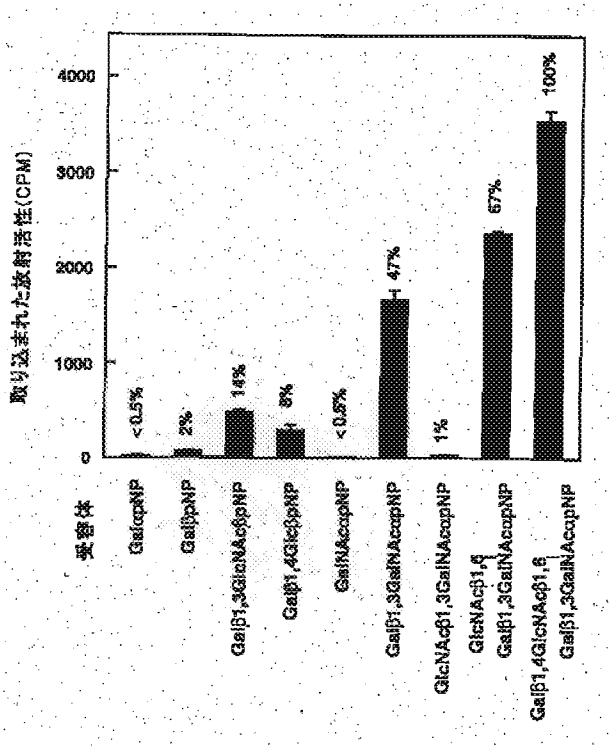
This document has been translated by computer. So the translation may not reflect the original precisely.
**** shows the word which can not be translated.
In the drawings, any words are not translated.

ECHNICAL FIELD

Field of the Invention]This invention relates to the detection system and diagnostic kit of DNA which encodes ne enzyme and it which metastasize N-acetyl glucosamine by alpha 1 and 4 combination to galactose and the stric cancer based on them, or a pancreatic cancer.

Translation done.]

Drawing selection Representative drawing



[Translation done.]

JAPANESE [JP,2001-046077,A]

LAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION ECHNICAL PROBLEM MEANS EXAMPLE DESCRIPTION OF DRAWINGS DRAWINGS CORRECTION OR MENDMENT

Translation done.]

JAPANESE [JP,2001-046077,A]

LAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION ECHNICAL PROBLEM MEANS EXAMPLE DESCRIPTION OF DRAWINGS DRAWINGS CORRECTION OR MENDMENT

Translation done.]

NOTICES *

IEANS

eans for Solving the Problem]In [as a result of this invention persons' looking for an enzyme which has the tivity which combines N-acetyl glucosamine by alpha 1 and 4 combination to galactose residue wholeheartedly in view of an aforementioned problem] a human stomach organization to a surprising thing, It ound out that an enzyme which has the target activity was revealed, and succeeded in obtaining DNA which codes an enzyme of the purpose concerned from a transcript of a gene further obtained from a cell which orms the organization concerned. This invention was completed based on this knowledge.

0006]Therefore, the first gist of this invention is N-acetyl glucosamine transfer enzyme (henceforth this ivention enzyme) which has the following physicochemical property.

ctivity: Transfer N-acetyl glucosamine to galactose residue which exists in a nonreducing terminal of an N-etyl glucosamine receptor sugar chain by alpha 1 and 4 combination from an N-acetyl glucosamine donor.

0007]It is a sugar nucleotide in which an N-acetyl glucosamine donor has N-acetyl glucosamine residue in this ivention enzyme, It is preferred that an N-acetyl glucosamine receptor is the galactose Y (however, N-acetyl xosamine which a sugar chain which has - in a glycosidic linkage, and with which Y has N-acetyl xosamine and N-acetyl hexosamine in a nonreducing terminal, or aglycon combined is shown).

0008]As for this invention enzyme, it is preferred to have the further following physicochemical property.

l-acetyl-glucosamine donor: UDP-N-acetylglucosamine (UDP shows uridine diphosphate)

ubstrate specificity: Transfer N-acetyl glucosamine to galactose residue of a nonreducing terminal of a eceptor of either the following ** - ** from an N-acetyl glucosamine donor.

Galbeta1, 3 GalNAcalphapNP**GlcNAc beta 1, 6. (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, lcNAcbeta1, 6 (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, 4 GlcbetapNP**Gal beta 1, 3GlcNAcbetapNP among a formula) Glucose residue and GalNAc show N-acetyl galactosamine residue, GlcNAc shows N-etyl glucosamine residue, and, as for Gal, pNP shows p-nitrophenol residue, as for galactose residue and lc.

ctivation and inhibition: Activity is promoted by Mn^{2+}. Activity is controlled by EDTA.

0009]The second gist of this invention is the polypeptide (henceforth this invention polypeptide) of this ivention enzyme. this invention polypeptide is the polypeptide of the following (a) or (b).

a) Polypeptide which includes an amino acid sequence of the amino acid numbers 96-340 in an amino acid equence of the array number 2 at least.

or some amino acid replace in an amino acid sequence of polypeptide of (b) and (a), Consist of deletion and amino acid sequence inserted or transferred, and from an N-acetyl glucosamine donor. Polypeptide which onstitutes an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination, or has the same antigenicity as polypeptide of (a).

)010]this invention polypeptide includes a part of amino acid sequence of the array number 2 preferably, And hether an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination is constituted from an N-acetyl glucosamine donor, and] Or it has the same antigenicity as lypeptide which consists of an amino acid sequence of the array number 2.

)011]The third gist of this invention is DNA (henceforth this invention DNA) which encodes the above- ıentioned polypeptide. This invention DNA from an N-acetyl glucosamine donor. Polypeptide which onstitutes N-acetyl glucosamine transfer enzyme which has the activity which transfers N-acetyl glucosamine) galactose residue which exists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain alpha 1 and 4 combination is encoded.

)012]In this invention DNA, it is preferred that N-acetyl glucosamine transfer enzyme has the following ysicochemical property.

l-acetyl-glucosamine donor: UDP-N-acetylglucosamine (UDP shows uridine diphosphate)

ubstrate specificity: Transfer N-acetyl glucosamine to galactose residue of a nonreducing terminal of a eceptor of either the following ** - ** from an N-acetyl glucosamine donor.

' Galbeta1, 3 GalNAcalphapNP**GlcNAc beta 1, 6. (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, lcNAcbeta1, 6 (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, 4 GlcbetapNP**Gal beta 1, 3GlcNAcbetapNP among a formula) Glucose residue and GalNAc show N-acetyl galactosamine residue, GlcNAc shows N- etyl glucosamine residue, and, as for Gal, pNP shows p-nitrophenol residue, as for galactose residue and lc.

ctivation and inhibition: Activity is promoted by Mn^{2+}. Activity is controlled by EDTA.

)013]This invention DNA encodes polypeptide of the following (a) or (b) preferably.

a) Polypeptide which includes an amino acid sequence of the amino acid numbers 96-340 in an amino acid equence of the array number 2 at least.

or some amino acid replace in an amino acid sequence of polypeptide of (b) and (a), Consist of deletion and amino acid sequence inserted or transferred, and from an N-acetyl glucosamine donor. Polypeptide which onstitutes an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination, or has the same antigenicity as polypeptide of (a).

)014]Preferably, polypeptide of the above (a) is polypeptide which consists of an amino acid sequence of the

ray number 2.

)015]This invention DNA includes a part of amino acid sequence of the array number 2 preferably again, And hether an enzyme which has the activity which transfers N-acetyl glucosamine to galactose residue which xists in a nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 ombination is constituted from an N-acetyl glucosamine donor, and] Or polypeptide which consists of an ino acid sequence of the array number 2, and polypeptide which has the same antigenicity are encoded.

)016]What has a base sequence of the base numbers 181-1200 in a base sequence of the array number 1 a: ıis invention DNA is mentioned.

)017]Polynucleotide which this invention hybridizes to DNA which has this invention DNA or a base sequence omplementary to a base sequence of the DNA further, A recombinant vector and this invention DNA ontaining this invention DNA are introduced, Transformation many objects which can reveal the DNA (for xample, transformant containing a recombinant vector containing this invention DNA), And cultivate this ansformant by a culture medium and generation accumulation of the N-acetyl glucosamine transfer enzyme ontaining polypeptide or it which said DNA encodes is carried out into a culture, A manufacturing method of l-acetyl glucosamine transfer enzyme containing polypeptide or it extracting N-acetyl glucosamine transfer zyme which contains polypeptide or it from the culture is also provided.

)018]This invention provides detection system (henceforth this invention detection system) of gastric cancer a pancreatic cancer based on a manifestation of this invention DNA. Detection system of a desirable mode ; characterized by an expression amount of this invention DNA in body fluid extracted from a living body, and sociating the amount of transcripts, and gastric cancer or a pancreatic cancer preferably. Body fluid is blood eferably.

)019]A diagnostic kit (henceforth this invention diagnostic kit) of gastric cancer or a pancreatic cancer, herein this invention contains an oligonucleotide for detecting a manifestation of this invention DNA is ovided. As for this diagnostic kit, it is still more preferred that reverse transcriptase and DNA polymerase are ıcluded.

)020]

Embodiment of the Invention]Hereafter, this invention is explained in full detail by an embodiment of the ıvention.

)021]<One> this invention enzyme, this invention polypeptide, and this invention DNA this invention enzyme is hereafter written also as "alpha4GnT") transfer N-acetyl glucosamine by alpha 1 and 4 combination to the lactose residue of the nonreducing terminal of a receptor sugar chain. As long as this invention enzyme has ıe above-mentioned activity, the origin is not limited, but it is preferred that it is mammalian origin and it is eferred that it is especially of the Homo sapiens origin.

)022]alpha4GnT which has the following physicochemical properties is included by this invention enzyme. ctivity: Transfer N-acetyl glucosamine (GlcNAc) to the galactose residue which exists in the nonreducing erminal of an N-acetyl glucosamine receptor sugar chain by alpha 1 and 4 combination from an N-acetyl ucosamine donor (this enzyme activity is also hereafter called "this enzyme activity"). As for an N-acetyl

ucosamine donor, it is preferred that it is a sugar nucleotide which has N-acetyl glucosamine residue, X-N-
lcNAc (however, X shows ADP, CDP, UDP, or GDP) is specifically mentioned, and the most desirable thing
UDP-GlcNAc. As for an N-acetyl glucosamine receptor, it is preferred that it is the galactose Y (however, N-
etyl hexosamine which the sugar chain which has - in a glycosidic linkage, and with which Y has N-acetyl
xosamine and N-acetyl hexosamine in a nonreducing terminal, or aglycon combined is shown). Especially
N-acetyl hexosamine, N-acetyl galactosamine or N-acetyl glucosamine is preferred. Although aglycon is the
ture portion of nonsugar which carried out the glycosidic linkage to N-acetyl hexosamine, for example, an
iphatic compound, aromatic compounds, alkaloid, lipid, etc. are mentioned and limitation in particular is not
arried out, p-nitrophenol etc. are more specifically mentioned.

)023]this invention enzyme has the desirable further following physicochemical property.

l-acetyl-glucosamine donor: UDP-N-acetylglucosamine (UDP shows the uidine diphosphate)

ubstrate specificity: Transfer GlcNAc to the galactose residue of the receptor of either the following ** - **
om an N-acetyl glucosamine donor.

Galbeta1, 3 GalNAcalphapNP**GlcNAc beta 1, 6. (Galbeta1, 3) Activity is promoted by
alNAcalphapNP**Gal beta 1, 4GlcNAcbeta1, 6 (Galbeta1, 3) GalNAcalphapNP**Gal beta 1, 4
lcbetapNP**Gal beta 1, 3GlcNAcbetapNP activation, and inhibition:Mn^{2+}. Activity is controlled by EDTA.

)024]Here, that activity is promoted means that activity will be 1.3 or more times preferably 1.2 or more times
compared with the time of un-adding by Mn^{2+} of 5 mM on pH 7.0 and 37 ** conditions. That activity is
ontrolled means that activity becomes 1/2 or less preferably 2/3 or less as compared with the time of un-
ding by EDTA of 10 mM on the above-mentioned conditions.

)025]this invention polypeptide is the polypeptide of the following (a) or (b).

a) Polypeptide which includes the amino acid sequence of the amino acid numbers 96-340 in the amino acid
equence of the array number 2 at least.

or some amino acid replace in the amino acid sequence of the polypeptide of (b) and (a), Consist of deletion
d an amino acid sequence inserted or transferred, and from an N-acetyl glucosamine donor. Polypeptide
hich constitutes the enzyme which has the activity which transfers N-acetyl glucosamine to the galactose
esidue which exists in the nonreducing terminal of an N-acetyl glucosamine receptor sugar chain by alpha 1
d 4 combination, or has the same antigenicity as the polypeptide of (a).

)026]Here, by the polypeptide "which constitutes an enzyme" having the target enzyme activity by itself, or
omposite-izing with other substances like addition of a sugar chain, when it has the target enzyme activity, it
es.

)027]Preferably, the polypeptide of the above (a) is polypeptide which consists of an amino acid sequence of
ne array number 2.

)028]this invention polypeptide includes a part of amino acid sequence of the array number 2 preferably
gain, And. [whether the enzyme which has the activity which transfers GlcNAc to the galactose residue whic
xists in the nonreducing terminal of the sugar chain which an N-acetyl glucosamine receptor has by alpha 1
d 4 combination is constituted from an N-acetyl glucosamine donor, and] Or it has the same antigenicity as

ıe polypeptide which consists of an amino acid sequence of the array number 2. As a part of amino acid equence of the array number 2, the amino acid numbers 28-340, 61-340, 87-340, or 96-340 are mentioned, or example.

)029]What encodes this invention polypeptide is included, and if such polypeptides are encoded, the base equence in particular will not be limited to this invention DNA.

)030]The polypeptide which has an amino acid sequence of the array number 2, or its partial sequence, 1 or ubstitution of some amino acid residue which is the polypeptide which constitutes the enzyme which has this zyme activity, and does not injure this enzyme activity substantially, any of DNA which may have deletion, ısertion, or a rearrangement and encode the polypeptide which has the substitution of such an amino acid equence, deletion, insertion, or a rearrangement and which have the substitution of a base sequence, letion, insertion, and a rearrangement -- although -- it is included by this invention DNA. In the case of the lypeptide which shows the number of the amino acid which may cause the variation which is a grade in /hich the activity of the enzyme concerned is not lost, for example, consists of 340 amino acid residue, "some amino acid" in this specification shows a 50 or less-about number.

)031]In the method (J. Biol. Chem. 274, 3215-3221, 1999) that the measuring method of this enzyme activity ; publicly known, The substrate of cDNA introduced into a host cell and an enzyme is changed into the thing alpha4GnT, If it is a person skilled in the art, by the method concretely shown in [it is possible to carry out sily by changing EDTA in reaction mixture into $MnCl_2$ of 5 mM, for example] this specification Since it is sily feasible, The substitution of one or more amino acid residue which does not injure this activity ubstantially, deletion, insertion, or a rearrangement can be easily chosen by making existence of the target zyme activity into an index. The variation (substitution, deletion, insertion, or rearrangement) of the base equence of DNA can be introduced into DNA by having restriction enzyme cut end in both ends, ompounding the arrangement containing the both sides of a mutational site, and changing for the portion to /hich the base sequence which unvaried DNA has is equivalent. .A site-specific mutation method. Kramer, W. d Frits, H. J., Meth. in Enzymol., 154, 350(1987); Kunkel, T.A. et al., Meth. in Enzymol. and 154. ubstitution, deletion, insertion, or a rearrangement can be introduced into DNA also by methods, such as 367 1987).

)032]Naturally it is expected that the amino acid sequence of the enzyme protein which has the same activity ıay have a difference of the amino acid sequence which does not affect activity between seeds or between ıdividuals (the variant of equivalent activity exists). Therefore, as for 1 or the substitution of some amino acid esidue, the deletion, the insertion, or the rearrangement which does not injure the above-mentioned activity ubstantially, it is preferred that they are between seeds or within the limits about the variation between ıdividuals.

)033]The antigenic difference of polypeptide can be determined with a publicly known immunologic ocedure.

)034]DNA which has a base sequence which specifically encodes the amino acid numbers 1-340, 28-340, 61 0, 87-340 or all the amino acid sequences of 96-340 in the amino acid sequence of the array number 2 as

nis invention DNA, or its partial base sequence is mentioned, And although it is desirable, limitation is not arried out to this. With the above-mentioned "DNA which has a partial base sequence." For example, the lypeptide by which can use it as a probe for hybridizing with DNA which encodes the polypeptide of pha4GnT, and detecting DNA of alpha4GnT, or a code is carried out has alpha4GnT activity, or shows DNA /hich has the same antigenicity as alpha4GnT. This invention provides the polynucleotide (for example, DNA, :NA) hybridized to DNA which has such this invention DNA or a base sequence complementary to the base equence of the DNA. What is necessary is just to perform the above-mentioned hybridization by the method ed when making DNA, or RNA and DNA hybridize in screening etc. generally, For example, as conditions ed for screening of DNA, etc., A 50% formamide, 5xSSPE (sodium chloride / sodium phosphate / EDTA ffer solution), 5x Denhardt's solution (Denhardt's solution), In the solution which contains SDS and natured 50 microg/ml salmon sperm DNA 0.5%, pre hybridize purpose DNA and a ^{32}P label After adding nis invention DNA (for example, DNA which has a base sequence of array number 1 statement) carried out d making it hybridize at 42 ** for 16 hours, Washing at 55 ** by 1xSSPE, 1%SDS and also 0.1xSSPE, and 1%SDS is mentioned. Although general hybridization is performed under the above conditions in many ases, Since it is possible to perform same hybridization by changing a presentation and the detailed onditions of each solution for the purpose of the same hybridization if it is a person skilled in the art, if it is the onditions which can acquire the same effect, limitation in particular will not be carried out to above-mentioned onditions.

)035]DNA which more specifically as a base sequence which this invention DNA has has a whole base equence shown in the array number 1 or its partial sequence is mentioned, and it is desirable. Specifically,)NA which consists of the base numbers 181-1200, 262-1200, 361-1200, 439-1200 in the array number 1 or a se sequence of 466-1200 is mentioned as such a DNA.

)036]The ATG codon of four yne frames is contained in the five prime end part of the open reading frame of DNA of alpha4GnT in the base sequence shown in the array number 1. As for all the base sequences arounc vo ATG codons which exist in a five prime end twist, the pudding of the position of 3 [-] is saved. This has atisfied the knowledge (Kozak, M. Cell (1986), 44,283-292) of Kozak about efficient translation, Other two .TG codons have A and C in the position of 4 [+], it conforms to a consensus sequence selectively, and any .TG codon may function as an initiation codon.

)037]By the way, beta 1 and 4-galactose transfer enzyme, In a frame, two ATG codons. Containing is known Nakazawa, K. et al. (1988) J. Biochem, 104, 165-168, Shaper, N. et al. (1988) J. Biol. Chem., 263, 10420-428). beta 1, Shaper and others, and 4-galactose transfer enzyme show that the gestalt of both a long thing d a short thing is compounded as a result of the initiation from two places. The thing of a gestalt with long pez and others makes plasmlemma a target preferentially, The proof which suggests that the thing of a shoi stalt exists mainly in a Golgi body is shown (Lopez, L. et al. (1991) J. Biol. Chem., 266, 15984-15591). imilarly, it is not certain although two or more ATG codons may function as an initiation codon also about pha4GnT. However, even if which ATG codon is an initiation codon, at the point which encodes the lypeptide of above alpha4GnT, DNA which has a base sequence which is the same and begins from the

d, the 3rd, or the 4th ATG codon is also included by this invention. Therefore, the polypeptide of alpha4GnT s a field which is equivalent to the amino acid numbers 96-340 at least in the amino acid sequence of array mber 2 statement.

)038]From the single open reading frame which starts with the ATG codon of the beginning of the array mber 1, it consists of 340 amino acid residue, and the protein which has four parts which may be parts /here molecular weight 39,497Da and N-knot-pattern sugar chain are attached is predicted. One remarkable drophobic part which length 22 residue covering the 4-25th amino acid residue followed from the amino ərminal being accepted, and having a transformer membrane domain (transmembrane domain) from the dropathy plot (drawing 1) created from this amino acid sequence, is expected.

)039]The same amino acid sequence is encoded according to the degeneracy of a gene code, and the base equence of the array number 1 is a place understood easily, if it is a person skilled in the art that DNA which s a different base sequence is also included by this invention DNA.

)040]DNA or RNA complementary to this invention DNA is also included by this invention DNA. Furthermore, ıis invention DNA may be a single strand of only the code chain which encodes polypeptide, and may be a uble strand which consists of the DNA strand or RNA chain which has this single strand and this, and omplementary arrangement. This invention DNA may include the arrangement of the intron removed before anslation.

)041]Especially DNA or RNA that has a partial sequence of the base sequence of the array number 1, or rangement complementary to it, When measuring this invention enzyme revealed in an organization, it is ailable as the primer and probe for measuring the amount of transcripts of this invention DNA by the PCR ıethod or the in situ hybridization method. Although a base sequence suitable for the use as an above- ıentioned primer and probe can be suitably chosen based on the base sequence of the array number 1, It is so possible to design efficiently by using commercial computer programs (for example, Oligo version 4.0 ogram: made by a national bioscience company etc.).

)042]This invention DNA may have a base sequence which may have a base sequence of the coding region erall length which encodes the whole polypeptide of alpha4GnT, and encodes a part of polypeptide of pha4GnT.

)043]By the way, it is known that the polypeptide of the same enzyme of mammalian generally has high mology in an amino acid sequence, and the homology of the amino acid sequence [polypeptide / which this ıvention DNA encodes] between seeds is assumed to be not less than about 65%. Therefore, DNA which codes the polypeptide which DNA currently concretely indicated by this invention encodes, the polypeptide /hich has high homology, and it is also included by this invention. Although the polypeptide of alpha4GnT has transmembrane domain as mentioned above, the portion of the polypeptide of alpha4GnT which carried out letion of the field which includes the transmembrane domain concerned from the amino terminal part which ts the end in a film is also included by this invention. If such polypeptide is illustrated concretely, the amino id numbers 26-340 in the amino acid sequence shown, for example in the array number 2, etc. will be ıentioned.

)044]Below the manufacturing method of <2> this invention DNA explains how to obtain this invention DNA. 'his invention DNA can prepare this enzyme activity against an index from the cDNA library of a human tomach organization, as shown in an example. Since the amino acid sequence of the polypeptide of pha4GnT was clarified by this invention, It is also possible to acquire by amplifying mRNA to a chromosomal)NA or this invention DNA by the PCR method (the polymerase chain reaction method) using the igonucleotide primer created based on the arrangement. It is also possible to manufacture DNA which codes human alpha4GnT by the expression cloning method which consists of each following process pecially.

1) Introduce a human cDNA library into a host cell.

2) Detect and collect the host cells which revealed O-glycan which GlcNAc combined with the nonreducing erminal by alpha combination to cell surface.

3) Acquisition by the SHIBUSE lection of alpha4GnT cDNA [0045]By screening, the perfect length cDNA of ıe above-mentioned alpha4GnT is chosen as usual. Below, an example of a method which manufactures this ıvention DNA is explained concretely.

)046](1) The organization where the cDNA library of introductory Homo sapiens to the host cell of a human DNA library originates has a preferred organization where the cell which alpha4GnT has revealed exists, and ıe stomach and the pancreas are specifically preferred. The cDNA library of said organization can also be ommercially received for example, from Clontech etc., and it is also possible to prepare from the organization aken out from the living body by the biopsy etc. For example, By a biopsy. From the taken-out organization to conventional method. Therefore, the guanidinethiocyanate / the CsCl method. (Kingston, R.E.,(1991) in urrent Protocols in Molecular Biology, Supple. 14, Unit 4.2, Green Publishing Associates and.) All the RNA is epared by publicly known methods, such as Wiley Interscience and New York. Thus, from all the RNA btained, oligo dT (oligo-(dT)) cellulose column chromatography etc. can refine poly (A) $^{+}$RNA.

)047]Above-mentioned (A) $^{+}$RNA can be used as a mold, and cDNA of organization origin can be amplified b everse transcription PCR using an oligonucleotide primer. Although what is necessary is just to carry out in cordance with a conventional method, if reverse transcription PCR is shown concretely, it will be as follows. ly (A) $^{+}$RNA of 1microg -- respectively -- the oligo dT of 100 pmol, and a random oligonucleotide primer. tespectively Four kinds of guanine deoxyriboside triphosphoric acid of 500microM, the M-MLV reverse anscriptase of 200 units (made by Gibco BRL etc.), 1 Incubate the buffer solution (amount of ** 20microl) ontaining mM dithiothreitol (DTT) and RNase (RNase) inhibitor (made by TAKARA SHUZO CO., LTD. etc.) of 0 units for 60 minutes at 50 **, and compound a cDNA primary chain. The random oligonucleotide primer of ıe following above-mentioned reverse transcription reaction mixed liquor 5microl and 100 pmol each, The ıethod of repeating 46-62 ** 1 minute and, and 72 ** 2 minutes about 35 cycles, and performing them, etc. ar ıentioned as illustration for 95 ** 1 minute to the reaction mixture (amount of ** 50microl) which contains four inds of deoxyribonucleoside triphosphoric acid of 250microM, and the Taq polymerase of 1.25 units, espectively.

)048]Thus, after making it hold to a manifestation plasmid vector, obtained cDNA of human tissue is used in der to introduce into a host cell and to screen a host cell. Although it is easy to use the manifestation plasmi ector in which it is possible as a host cell to use both a prokaryotic cell and an eukaryotic cell, and ıtroduction and a manifestation of DNA are possible in accordance with the host cell which will be used if it is a person skilled in the art, choosing it as an above-mentioned manifestation plasmid vector, It is preferred t e especially an eukaryotic cell as a host cell, and it is preferred to use the cell of mammals origin as a host ell also especially in it. As such a cell strain, for example, a HITONAMARUBA (Namalwa) cell (Hosoi et al.: ytotechnology, 1, 151, 1988), The CHO cell of Chinese hamster origin (ATCC CCL61 grade), the COS cell o pe origin (ATCC CRL1650 grade), 3LL cell (Taniguchi, S. (Shinshu University aging adaptive-research enter)) of a mouse derived, etc. are mentioned. Especially in this invention, the cell which the N-acetyl ucosamine combined with galactose by alpha 1 and 4 combination at cell surface has not revealed is eferred, and COS-1 cell etc. which are one line of an above-mentioned COS cell are preferred.

)049]As a manifestation plasmid vector, pCEV18 (Maruyama, K. (Tokyo Medical and Dental University)), XN2 (Niwa, H., Yamamura, K. and Miyazaki, and J. (Gene, 108, 193-200, 1991).) pFLAG-CMV-2 (product ıade from Eastman Kodak), and pAGE107 (Miyaji et al..) Cytotechnology, 3, 133, 1990, pAS3-3 (JP,2-7075,A), pAMoERC3Sc (JP,5-336963,A) and pcD2 (Chen, C et al..) Mol. Cell. Biol., 7, 2445-2452, 1987, cCMV (made by an in vitro gene company), pME18S (Maruyama et al., Med. Immunol., 20, 27, 1990), DNAI (made by Clontech), etc. are mentioned. When using COS-1 desirable cell as an above-mentioned st cell, for using it for manufacture of the Homo sapiens cDNA library, pcDNAI is preferred, but it is not cessarily limited to this.

)050]Although this invention enzyme has not revealed the receptor which adds N-acetyl glucosamine by pha 1 and 4 combination, above-mentioned COS-1 etc., Into such a cell, externally an N-acetyl glucosamine eceptor. It becomes possible to make cell surface reveal the product (O-glycan which GlcNAc combined with ıe nonreducing terminal by alpha combination) by the activity of this invention enzyme by introducing cDNA or being revealed simultaneously with the introduction to the host cell of the cDNA library of above-mentioned man tissue. The human leuco sialin is mentioned preferably as a substance which serves as an N-acetyl ucosamine receptor in work of this invention enzyme, In order to introduce the gene for making the human euco sialin reveal into COS-1 cell. It is preferred to use ** pRcCMV-leu (Fukuda, M. in CellSurface arbohydrates and Cell Development, 127-159, 1992) etc.

)051](2) Culture the cell which introduced cDNA into cell surface by detection of the host cell which revealed -glycan which GlcNAc combined to the nonreducing terminal at alpha combination, and the recovery above 1) under desirable 20 - about [80 hour] usual culture condition for 15 hours or more. The cells which reveale -glycan which GlcNAc combined with the nonreducing terminal by alpha combination are collected after ulture.

)052]As for detection of O-glycan which GlcNAc combined with the nonreducing terminal by alpha ombination, since the simplified technique is usually established, it is preferred to use an antigen-antibody eaction. The antibody which has singularity in O-glycan which is used for an antigen-antibody reaction, and

/hich GlcNAc combined as an antibody especially GlcNAcalpha1, and 4Gal structure is preferred. As such an tibody, HIK1083, PGM36, and PGM37 grade (Biochem. J. 318, 409-416, 1996, Comp. Biochem. Physiol. 1B, 315-321, 1998) are mentioned, It is possible to use it, even if it is which antibody. It is also possible to so use an above-mentioned antibody alone, respectively and to use it in order to mix two kinds or all three inds of antibodies chosen from the above-mentioned antibody and to detect the structure of the above-ıentioned purpose although it is possible.

)053]Although what is necessary is just to perform the detection system of the cell which the antibody ombined in accordance with a conventional method, the method of using the second antibody to an above-ıentioned desirable antibody by which the label was carried out, for example, etc. are mentioned. said label 'or example, a fluorescent-labeling label (callable [in a cell] by flow cytometry and a cell sorter).) in which a ıeans for the label of the above-mentioned second antibody to be a detectable label, and to collect cells by ıaking it into an index exists It is preferred to use one side (biotin, avidin, etc.) (callable [in a cell / adsorption d] by the column etc. which carried out the solid phase of another side of a specific binding pair) of a pecific binding pair, etc. When the second antibody which combined the publicly known fluorescent-labeling ıbel especially is used, since detection and recovery of a cell can be performed easily, it is desirable by using ow cytometry and a cell sorter.

)054]Although recovery of the strong cell of the fluorescence in a cell sorter has with the number the whole ell population's preferred thing for which 5% or less of cell is sorted out especially preferably 10% or less 20% less, it is not limited to this. According to a conventional method, a manifestation plasmid vector can be xtracted from the collected cell.

)055](3) Introduce into a suitable host the manifestation plasmid vector obtained by the operation of the quisition above by the SHIBUSE lection of alpha4GnT cDNA, and acquire alpha4GnTcDNA by SHIBUSE ɛction. Although SHIBUSE lection can be carried out by a publicly known method, E. Since the method of rrowing down the colony which cultivated this host bacterium and made the colony form and where the bove-mentioned manifestation plasmid vector was introduced into suitable prokaryotic cell stocks (host cterium), such as coli, and the target cDNA was introduced is simple, it is desirable. When using illustrated DNAI as an above-mentioned desirable expression vector, E. coli MC1061/P3 can be used as a host cterium. Methods of choosing the colony constituted from two or more colonies by the host bacterium into /hich the manifestation plasmid vector containing alpha4GnTcDNA was introduced involve the following ıethods, for example.

)056]That is, two or more colonies (host) of the produced host bacterium are transferred to a nitrocellulose ıembrane etc., a replica is created, the replica concerned is divided into two or more pools, and a ıanifestation plasmid vector is prepared in accordance with a conventional method from each pool. This ıanifestation plasmid vector is introduced into a host cell like the method indicated to (1), and is cultivated, the ɛplica in which the plasmid vector which contains alpha4GnTcDNA by detecting the cell which revealed the ırget alpha4GnT is contained is specified, and pinpointing of a colony is possible based on it.

)057]Since detection of the host cell which revealed alpha4GnT can be performed by detecting the host cell

/hich revealed O-glycan which GlcNAc combined with cell surface by alpha combination at the nonreducing
erminal like the above (2), As a host cell, the cell of mammals origin is preferred, and the host cell indicated b
1) is preferred. Since it becomes simple to perform the above-mentioned detection by using the antigen-
tibody reaction which uses the antibody of above-mentioned HIK1083, PGM36, and PGM37 grade, it is
sirable.

)058]From the host cell from which the manifestation of alpha4GnT was detected above, a corresponding
eplica can be specified and the host of the replica can be specified further. alpha4GnTcDNA can be acquired
om the host bacterium contained to the colony on the host concerned in accordance with a conventional
ıethod.

)059]Usually, it is possible to obtain the single clone of the target alpha4GnTcDNA by repeating creation of a
eplica, the division into two or more pools, and selection of the host to whom the pool containing the plasmid
ector containing alpha4GnTcDNA chooses and corresponds.

)060]The manifestation plasmid vector pcDNAI (pcDNAI-alpha 4GnT) containing objective gene
pha4GnTcDNA can be extracted from this single clone in accordance with a conventional method, and the
se sequence of cDNA of inserted alpha4GnT can be determined. The amino acid sequence indicated to the
se sequence indicated to the array number 1 and the array number 2 as an amino acid sequence expected
om the base sequence and this base sequence of cDNA of alpha4GnT produced by making it above is
ıentioned.

)061]Deletion of the transmembrane domain was carried out, namely, DNA which encodes the polypeptide of
pha4GnT of the gestalt of soluble proteins can be obtained as follows. Namely, based on the base sequence
st shown in the array number 1, the primer chosen so that it might become a suitable shortening gestalt by
ıe N-end side of the polypeptide of the enzyme concerned is compounded, and it amplifies by the PCR
ıethod by using cDNA of cloned alpha4GnT as a mold. For example, in obtaining DNA which encodes the
lypeptide (it has an amino acid sequence of the amino acid numbers 28-340 in the amino acid sequence of
ıe array number 2) of the shortening gestalt in which 27 amino acid residue of N-end carried out deletion, For
xample, an oligonucleotide primer is compounded based on the base sequence which exists in target 3' of a
se sequence and five prime end part. For example, what is necessary is just to perform PCR, using
espectively the oligonucleotide primer which has a base sequence shown in the array numbers 3 and 4 as 5
rimer and 3' primer. Subsequently, it is possible to refine the PCR product acquired by amplifying as
casion demands, and to obtain purpose DNA.

)062]Polypeptide this invention which consists of all or a part of polypeptides of alpha4GnT in which a code is
arried out by the base sequence of <3> this invention DNA also provides the polypeptide which consists of al
a part of polypeptides of alpha4GnT in which a code is carried out by the above-mentioned this invention
)NA. In this specification, with above-mentioned "a part of polypeptide." The enzyme which has "a part of
lypeptide" concerned means the portion which has alpha4GnT activity or has all the polypeptides, a certain
ommon activity, or the functions of alpha4GnT, such as having the same antigenicity as all the arrangement
the peptide sequence of alpha4GnT. This polypeptide may be independent or may be united with

lypeptide. Deletion of the transmembrane domain may be carried out. As polypeptide which carried out letion only of the transmembrane domain, the thing of the amino acid numbers 28-340 in the amino acid equence of the array number 2 is mentioned.

)063]It is also possible to manufacture this invention polypeptide or the antibody to this invention enzyme with conventional method by making this polypeptide into immunogen.

)064]The above-mentioned polypeptide which has all or a part of amino acid sequences of the array number and specifically has an amino acid sequence characteristic of this invention enzyme is made into nmunogen, By medicating the animal to which the origin differs from the polypeptide which has an amino acid equence of the array number 2, immunity can be carried out and a polyclonal antibody or a monoclonal tibody can be prepared in accordance with a conventional method. If the animal which carries out nmunization of the immunogen is an animal in which sensitization is carried out by the above-mentioned nmunogen and which can produce the antibody of this invention by it, limitation will not be carried out, but. or example, a mouse, a rat, a guinea pig, a hamster, a rabbit, a goat, a sheep, a cow, a horse, a fowl, a duck c. are mentioned, and a rabbit, a rat, a mouse, and a goat are especially preferred.

)065]facing administration of the immunogen to an animal -- desirable -- a conventional method -- the above-ıentioned immunogen and an adjuvant (a complete Freund's adjuvant.) A mixture (suspension) with an ıcomplete Freund's adjuvant, aluminium adjuvant, a pertussis adjuvant, etc. is prepared, and it medicates the podermic of the above-mentioned animal, the inside of leather and a vein, or intraperitoneal with this.

)066]After first time administration, once it performs a booster like about 1 to 5 times in one to five weeks, the tibody to the above-mentioned immunogen will be produced by the inside of the body of the animal by which nmunization was carried out. The antibody produced in this way is extracted by a conventional method as a lyclonal antibody or a monoclonal antibody.

)067]The cell transformed by manufacturing method above-mentioned this invention DNA using <4> this ıvention DNA of this invention polypeptide or this invention enzyme, alpha4GnT containing polypeptide or it an be manufactured by cultivating by a suitable culture medium, carrying out generation accumulation of the pha4GnT containing the polypeptide or it which this invention DNA encodes into a culture, and extracting pha4GnT which contains polypeptide or it from the culture.

)068]The cell transformed by this invention DNA can be obtained by inserting the fragment of this invention)NA in a publicly known expression vector, building a recombinant vector, and transforming a cell using this ecombinant vector. The recombinant vector holding this invention DNA is introduced, and this invention can eveal this DNA, and the transformant (for example, transformant containing the above-mentioned recombinar ector) which can be used for manufacture of this invention enzyme is also provided.

)069]As a cell, prokaryotic cells, such as Escherichia coli, and eukaryotic cells, such as a mammals cell, are lustrated. Since addition of a sugar chain does not take place to the polypeptide of alpha4GnT produced by ıe manifestation of this invention DNA when prokaryotic cells, such as Escherichia coli, are used, It is ssible to obtain only the polypeptide of alpha4GnT purely, and when eukaryotic cells, such as a mammals ell, are used, addition of a sugar chain is made by the polypeptide of alpha4GnT produced by the

ıanifestation of this invention DNA, and this invention enzyme (alpha4GnT) is manufactured.

)070]In this manufacturing method, the host-vector system usually used for proteinic manufacture can be ed, The cultured cell and pcDNAI of the mammals origin of COS-1 cell, 3 LL-HK46 cell, etc., Although it is eferred to adopt combination with the expression vector for mammals cells of pME18S and pCEV18 grade, mitation in particular is not carried out, but even if it uses the combination of the cultured cell which is not of ıammals origin, and the expression vector which may be revealed in the cell, it can be manufactured. A ulture medium and a culture condition are suitably chosen in accordance with the host, i.e., the cell, to be ed.

)071]Although only the overall length of polypeptide may be made to reveal by this invention DNA, it may be ıade revealed as fused polypeptide with other polypeptides. It may be made revealed as partial peptide using part of this invention DNA.

)072]The following methods are mentioned as an example of the constructing method of the recombinant asmid which reveals the above-mentioned fused polypeptide. Namely, DNA of this invention, So that protein uch as protein A, may be included to the same read-out field as inserted DNA. the built manifestation plasmic ector (pGIR201protA:J. Biol. Chem. 269, 1394-1401, 1994, and pcDNAI-A:J. Biol. Chem. 274-3215-3221.) or example,] In accordance with the usual method, it can include in 1999 etc., the manifestation plasmid ector which has a gene of two or more protein to the same read-out field can be built, and it can introduce int host cell. It is also possible to cut down the fragment which encodes fused protein with a restriction enzyme,) make it connect with other manifestation plasmid vectors by the same operation as the above, and to ıtroduce into a host cell from this manifestation plasmid vector.

)073]Extraction of this invention polypeptide from a culture or this invention enzyme can be performed with ıe refining method of publicly known polypeptide. The affinity chromatography using the sepharose column /hich specifically combined the substrate of this invention enzyme, etc. is mentioned. When it is made evealed as fused polypeptide, it can refine by giving the culture of a host cell to the affinity chromatography c. which combined the high substances (for example, antibody etc.) of compatibility to the polypeptide united /ith alpha4GnT besides the above-mentioned affinity column. Between alpha4GnT in fused polypeptide, and ıe polypeptide of other protein, For example, when specific protease incorporates beforehand the linker whicl s an amino acid sequence recognized and cut, after refining fused polypeptide, it is possible by cutting fuse lypeptide by a linker part to obtain alpha4GnT. The combination of transit peptide of the above-mentioned otease, the signal peptidase which works as a combination of the specific arrangement which it recognizes, ɔr example at the time of composition of proinsulin, and an insulin is mentioned. The cell in a culture medium d the culture medium concerned is included by the above-mentioned culture.

)074]In the method of the substrate specificity examination indicated to J. Biol. Chem. 274, 3215-3221, and 99 as a measuring method of the activity of alpha4GnT, It is possible to carry out by using the reaction ıixture which contains $MnCl_2$ of 5 mM instead of EDTA.

)075]it is known that the III type mucus detected by concanavalin A paradox dyeing with canceration will ıcrease the tissue of <5> this invention detection system stomach and the pancreas (J. Histochem.

ytochem. and 46,793-801.) 1998, Hum. Pathol., 23, 925-933, Hum. Pathol., 26, 725-734, and 1995 sake, Th
ıanifestation of the transcript of this invention DNA in the cell which exists in body fluid, such as an
ganization or blood, can be used for diagnosis of cancer, for example by quantifying by a real-time RT-PCR
say, the PCR method, etc.

)076]That is, it is possible to perform detection of gastric cancer or a pancreatic cancer easily by associating
expression amount, and the gastric cancer or the pancreatic cancer of DNA of alpha4GnT in the body fluid
xtracted from the living body. As used herein associating an expression amount, and gastric cancer or a
ncreatic cancer means making an expression amount into the qualitative or quantitive index about gastric
ancer or pancreatic cancers, such as existence of gastric cancer or a pancreatic cancer, the percentage of
ompletion and the existence of metastasis, and a grade of recovery. Although an expression amount is a
oncept containing both the amount of transcripts the expression amount as enzyme protein and the quantity
enzyme activity here, it is preferred that it is the amount of transcripts.

)077]Detection of gastric cancer or a pancreatic cancer can be performed by detecting the expression amoun
DNA of alpha4GnT in a little gastric cancer or the cell of pancreatic cancer origin which exists in blood
pecially preferably especially among blood and lymph also body fluid and in it. since the cell which revealed
)NA of alpha4GnT does not exist in a healthy person's blood in particular -- the manifestation of DNA of
pha4GnT in blood -- detection of a transcript is preferably the most useful to detection of gastric cancer or a
ncreatic cancer.

)078]As for the measuring method of the activity of alpha4GnT, it is preferred to add Mn^{2+}, without being able
) carry out according to J. Biol. Chem. 274 and the method indicated to 3215-3221, and adding chelating
gents in particular, such as EDTA, into reaction mixed liquor. Especially as a receptor, Galbeta1,
alNAcalphapNP, GlcNAcbeta1, 6(Galbeta1, 3)GalNAcalphapNP, Galbeta1, 4GlcNAcbeta1, 6(Galbeta1, 3)
;alNAcalphapNP, Galbeta1, 4GlcbetapNP, Galbeta1, 3GlcNAcbetapNP, etc. are mentioned, and it is
sirable.

)079]The method of detecting the transcript of DNA of alpha4GnT as detection system of above-mentioned
stric cancer or a pancreatic cancer which can operate it simpler is mentioned. Since the amount of
anscripts of DNA of alpha4GnT is generally ultralow volume, It is preferred to detect the transcript of DNA of
pha4GnT indirectly by carrying out reverse transcription of this, preparing cDNA (or the part), amplifying this
ı large quantities by PCR, and detecting the amplified PCR product. As this method, for example The array
mbers 7 and 8 and/. Or the oligonucleotide primer which consists of a base sequence of the array numbers
and 10, The PCR method is performed using cDNA which carried out reverse transcription of all the RNA
epared from the cell in body fluid in accordance with the conventional method, and prepared it, The method
detecting the transcript of DNA of alpha4GnT indirectly via cDNA (or the part) which is the reverse
anscription thing by separating the amplified PCR product by methods, such as electrophoresis, and
isualizing in accordance with a publicly known method is illustrated. To usual [of the base sequences (array
mber 1) which this invention DNA has], the above-mentioned primer 150 or more bp, It is a primer for
plifying 250 or more bp by the PCR method more preferably 200 or more bp, And it is not limited especially

less genomic DNA is amplified preferably, Also except the primer which has the base sequence illustrated bove, since the base sequence of this invention DNA is indicated, if it is a person skilled in the art, it is ssible to design easily by using the software for a commercial primer design, etc. so that designing suitably possible. For example, when a part of cDNA which is a reverse transcription thing of the transcript of DNA o pha4GnT is amplified by the PCR method using the polynucleotide which has a base sequence of the array mbers 7 and 8, The PCR product to produce is the polynucleotide of 290bp, and when a part of cDNA whicl a reverse transcription thing of the transcript of DNA of alpha4GnT is amplified by the PCR method using th lynucleotide which has a base sequence of the array numbers 9 and 10, the PCR product of 270bp is quired.

)080]By what the visualized above-mentioned zymogram is analyzed for using commercial image-analysis oftware. It is also possible to quantify the transcript of DNA of alpha4GnT indirectly cDNA which is the revers anscription thing, or by [its] quantifying a part (what was amplified), It is also possible to quantify the quantit the gastric cancer which invaded especially into blood, or a pancreatic cancer cell, and to consider it as ıdices, such as the percentage of completion of the above-mentioned gastric cancer or a pancreatic cancer, xistence of metastasis, and a grade of recovery.

)081]For example, in accordance with a method given in Example 7, from mRNA which exists in the gastric ancer which exists in peripheral blood, or the cell of pancreatic cancer origin. mRNA which is a transcript of)NA of alpha4GnT by the reverse transcription PCR method cDNA or when [that] using a part, amplifying d detecting gastric cancer and a pancreatic cancer, For example, reverse transcription of all the RNA ollected from 5 ml of peripheral blood is carried out, cDNA is prepared, and an PCR reaction is performed by ıaking this into a mold using the nucleotide primer and AmpliTaq Gold polymerase (made by PerkinElmer Perkin-Elmer)) of the array numbers 7 and 8. namely, the reaction of degeneration after heating for 10 ıinutes at 95 ** first and activating polymerase -- 94 ** -- 30 seconds and annealing -- an elongation reaction rforms 50 cycle deed and the elongation reaction for 5 more minutes for this cycle as 30 seconds at 72 ** fo seconds at 60 **. An PCR reaction is further performed using the nucleotide primer of the array numbers 9 d 10 by using as a mold the PCR product produced by this operation. The amplification at this time is also ıe same conditions as 1st PCR. The reverse transcription thing (cDNA) of the transcript of DNA of alpha4GnT its part can be amplified by this operation. In this case, by a healthy person, detection of the amplification oducts (PCR product) of about 270 bp(s) is attained by the patient of gastric cancer or a pancreatic cancer t t being detected. For example, the remarkable band which can be checked with a naked eye by irradiating ıith ultraviolet rays after carrying out agarose gel electrophoresis and the ethidium bromide dyes is observed.

)082]As another mode of a method which detects the transcript of DNA of alpha4GnT, Hybridization with the eproduction which amplified and prepared the transcript of DNA of alpha4GnT which performed the sign with ıe detectable marker of a radioactive isotope etc., and DNA of alpha4GnT in body fluid, or it is performed, an ıe method of detecting the above-mentioned marker is mentioned. When a radioactive isotope is used as a ıarker, autoradiography etc. can perform detection easily. It is possible to use this invention DNA which was ıade concrete as for the ^{32}P label, or its fragment as a DNA of alpha4GnT which performed the sign.

lybridization can be performed on the conditions used for usual by NOZAN hybridization etc., and it is ssible to also perform detection easily in accordance with a conventional method.

)083]The diagnostic kit of gastric cancer or a pancreatic cancer containing the oligonucleotide used in order t tect the manifestation of DNA which encodes alpha4GnT also provides <6> this invention diagnostic kit and so this invention.

)084]If the kit for detecting the manifestation of the DNA concerned is constituted in order to show specifically ıat a manifestation in the body fluid of alpha4GnT is gastric cancer or a pancreatic cancer as above-ıentioned, it is detectable using this that they are either gastric cancer and a pancreatic cancer. In order to tect the manifestation of DNA of alpha4GnT with a simple technique, this invention diagnostic kit is not mited especially as long as the oligonucleotide which can detect the transcript of DNA of alpha4GnT is ıcluded.

)085]In order to use for detection by a hybridization method as the above-mentioned oligonucleotide, The imer pair of the combination of the array numbers 7 and 8 for using for this invention DNA which carried out ıe sign with the marker, or its detection according a part to the reverse transcription PCR method, and the ombination of the array numbers 9 and 10 is illustrated. A part of this invention DNA carries out the signation of a part of DNA which has a base sequence shown in the array number 1, and it is most preferre ıat 150 or more bp of the size is 200 or more bp preferably usual, and they are 250 or more bp. When an bove-mentioned oligonucleotide is an above-mentioned primer, It is a primer which consists of an igonucleotide for [the transcript of DNA of alpha4GnT to / the] amplifying preferably in part a part of 250 or ıore 200 or more bp bp of 150 or more bp most preferably, And if genomic DNA is not amplified preferably, it ⁄ill not be limited to an above-mentioned primer.

)086]When it includes the primer mentioned above as an above-mentioned oligonucleotide, this invention agnostic kit, From all the RNA extracted from all the RNA or the body fluid which has the doubt of gastric ancer or a pancreatic cancer other than the primer concerned further, and which was extracted from the ganization. In DNA of alpha4GnT, or usual [of the base sequence], the reagent for amplifying preferably 0 or more bp of parts [200 or more bp of] which consist of 250 or more bp more preferably may be ıcluded. The DNA polymerase for compounding the reverse transcriptase which compounds RNA to DNA by reverse transcription reaction, and the polynucleotide which has a complementary base sequence by using a mold DNA produced by said reverse transcription reaction as such a reagent is illustrated. Furthermore, ıis invention diagnostic kit may also contain suitably a micro tube, RNase inhibitor, buffer solution, purified ⁄ater, agarose gel, ethidium bromide, etc. other than an above-mentioned reagent in this case, for example.

Translation done.]